A Study on the Plasma Biochemical Indices of Heat-Stressed Broilers

H. Lin*, R. Du[1], X. H. Gu[1], F. C. Li and Z. Y. Zhang[1]
Department of Animal Science, Shandong Agricultural University, Taian, Shandong 271018, P. R. China

ABSTRACT : Four experiments were conducted to evaluate the effect of temperature and humidity on biochemical indices of Arbor Acres broilers at different weeks of age. The alkaline phosphatase (AKP), acid phosphatase (ACP), lactic dehydrogenase (LD), creatine kinase (CK), plasma glucose (Glu), calcium (Ca), potassium (K), chloride (Cl), urea nitrogen (UN), uric acid (UA), plasma thyroxin (T4), triiodothyronine (T3) and insulin levels were determined in all the four experiments. In experiment 1, the plasma Glu, LD and CK levels were increased by heat exposure (35℃, and 35, 60, or 85% RH, 2 h) and this effect was aggravated by longer exposure (24 h). No significant changes ($p>0.05$) were found in Ca concentration, activity of AKP and ACP. In experiment 2, temperature (10, 20, 30, 33℃) had significant effect on the levels of K, Cl, UN, UA levels and the activity of LD ($p<0.01$), but had no significant influence on the activity of CK ($p>0.05$). The UN, UK and LD levels were elevated by low temperature (10℃) ($p<0.01$), Cl content was increased by high temperature (33℃) ($p<0.01$), and K level was decreased by high (33℃) or low (10℃) temperature and increased by medium temperature (30℃) ($p<0.01$). The humidity (35, 85% RH) only had significant effect on Cl concentration which was decreased by high humidity ($p<0.01$). In experiment 3, the result showed that only the LD and CK activity were significantly increased ($p<0.01$) by high temperature (7, 24, 28, 32℃) or high humidity (35, 85% RH). Temperature and humidity had no significant effect on K, Cl, UA, UN and Glu levels ($p>0.05$). In experiment 4 (24, 27, 30, 33℃; 30, 45, 60, 75, 90% RH), plasma T3 level was declined by high temperature (33℃), and this phenomena disappeared in birds under high temperature and high humidity environment. T4 concentration in plasma was not affected by temperature ($p>0.05$), but was increased by high or low humidity ($p<0.01$). Neither temperature nor humidity had significant effect on plasma insulin concentration ($p>0.05$). The results of the four experiments suggested that broilers at different growth periods might have different thermal requirements and would response differently to heat exposure. The plasma biochemical indices themselves had big variation; the reaction of the indices to thermal exposure treatment differed with the age of broilers. The big variation of biochemical indices themselves might cover the response of indices to temperature and humidity treatments. *(Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 9 : 1210-1218)*

Key Words : Heat Stress, Temperature, Humidity, Biochemical Indices, Broiler

INTRODUCTION

The physiological characteristics of birds are thick feather covered, no sweat gland in the skin, and high body temperature. The harmful effect of high temperature has been well evaluated by researchers. The main path of heat dissemination for birds under hot environment is respiratory evaporation (Hillman et al., 1985). When air temperature rise, the breath frequency increase (Egbunike, 1979; Hassan and AL-Rawl, 1982; Raup and Bottje, 1990), and the evaporative heat loss increases significantly (Spiers, 1983; Chwalibog and Eggum, 1989; Wiernusz and Teeter, 1993). Nichelman et al. (1991) showed that the evaporative heat loss was suppressed significantly by high humidity. Chwalibog and Eggum (1989) reported that evaporative heat loss increased with the rise of temperature and decreased with the rise of humidity. As humidity could affect evaporate heat dissemination, the rise or decline of humidity would aggravate or alleviate the affection of heat stress, so the influence of humidity on temperature requirement is not known well.

The blood is functioned as the carrier of nutrients, metabolic wastes, and the pathway of humoral transmission. So the blood biochemical parameters would reflect the physiological state of body. The harmful effects of high temperature on the performance of birds have been well studied and more attention on the responses of blood biochemistry induces are paid by many researchers. Donkoh (1989) reported that the haematocrit, haematoglobin and total protein were declined by high temperature. Ostrowski-Meissner (1981) reported that the concentrations of plasma free amino acids and essential amino acids in birds suffered acute heat stress were decreased, but the uric acid level was increased. Lin (1988) reported that the levels of free fatty acid and blood glucose were increased in heat-stressed laying hens. It is also reported that, for birds under high temperature, the elevated activities of lactic dehydrogenase (Ostrowski-Meissner, 1981; Sharma and Gangwar, 1986), alkaline phosphatase (Vysotskaya et al., 1979), creatine kinase (Hocking et al., 1994), high concentration of corticosterone (Deyhim and Teeter, 1991; Du and Gu, 1995) and triiodothyronine (T3) (Yang, 1995). However, there are many different results about the

* Corresponding Author: H. Lin. Tel: +86-538-8241103, Fax: +86-538-8241419, E-mail: hailin@sadu.edu.cn.
[1] Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100094, P. R. China.
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blood biochemistry indices. The uncertainty of the parameters might cause confusion in the study of biochemistry and physiology response of heat-stressed birds. There are many other factors that might influence the reaction of plasma indices. Sharma and Ganwar (1986) reported that the activity of acid phosphatase (ACP) raised and alkaline phosphatase (AKP) declined along with the increase of age, respectively. On the other hand, the thermal requirement of broilers changes at different growth periods (Meltzer et al., 1982; Meltzer, 1983). So the reaction of broilers to thermal environment would change.

Although there are many studies conducted to evaluate the effects of air temperature, the effect of humidity have been overlook and research works have been seldom conducted. In the present study, four experiments were conducted to evaluate the effect of humidity and the comprehensive effects of air temperature and humidity on the blood biochemical indices of heat-stressed broilers and to investigate the sensitivity and certainty of different blood indices.

MATERIALS AND METHODS

Experiment animals

In all the four experiments, Arbor Acres broilers were used as experimental animal. The birds in each experiment were reared in normal environment (21± 1℃, 50-70% RH) at 24 h light regime. The birds were fed ad libitum with the diet formulated according to the recommendation of NRC (1994) (table 1). During experimental period, the experimental birds were free access to feed and water.

Experimental design

In Experiment 1, forty broilers of 3-wk of age were divided into four groups according to sex and body weight. The average body weight was 1,050± 127 g. When the experiment began, the birds were exposed to four kinds of thermal environment: 21℃ & 60% RH, 35℃ & 35% RH, 35℃ & 60% RH, 35℃ & 85% RH, in four climate chamber, respectively.

In Experiment 2 and 3, 80 3-wk-old birds with average body weight of 920.8 g and 80 7-wk-old birds weighting 2,043.0 g were used, respectively. The 80 birds in each experiment were sorted to five groups. The two experiments were designed as 4×2 factorial. The temperature treatments were 10, 25, 30, and 33℃; humidity treatments were 35 and 85% RH respectively in Experiment 2. In Experiment 3, the temperature treatments were 7, 24, 28, and 32℃; humidity treatments were 35% RH and 85% RH, respectively.

Two hundreds broilers of 6 wk of age were assigned to twenty groups according to body weight and sex, and to produce a 4×5 factorial experiment in Experiment 4. The temperature regimes were 24, 27, 30, and 33℃, respectively. The humidity treatments were 30, 45, 60, 75 and 90% RH, respectively.

Table 1. The composition of experimental diets

Ingredients	3-5 wk	6-8 wk
	--------- % ---------	
Corn	57.09	64.88
Soybean	30.89	24.53
Fish meal	3.0	3.0
Bone meal	1.0	1.0
Stone meal	1.15	1.0
Salt	0.15	0.12
DL-methionine	0.09	0.03
Mineral and vitamins premix	1.0[a]	1.0[b]
Plant oil	5.72	4.44
Calculated analysis;		
ME, MJ/kg	13.41	13.40
Crude protein, %	20.0	18.0
Met+Cys, %	0.72	0.60
Lys, %	1.03	0.90
Ca, %	0.90	0.83
EP, %	0.37	0.36

[a] Mineral and vitamin premix provided followings per kg of diet: manganese, 100 mg; zinc, 75 mg; iron, 80 mg; iodine, 0.65 mg; copper, 80 mg; selenium, 0.35 mg; retinyl acetate, 0.009 MIU; cholecalciferel, 0.002 MIU; vitamin E, 0.011 MIU; vitamin K, 1.0 mg; thiamin, 1.2 mg; riboflavin, 5.8 mg; niacin, 66mg; pantothenic acid, 10 mg; pyridoxine, 2.6 mg; biotin, 0.10 mg; folic acid, 0.7 mg; vitamin B_{12}, 0.012 mg; choline chloride, 400 mg.

[b] Mineral and vitamin premix provided followings per kg of diet: manganese, 100 mg; zinc, 75 mg; iron, 80 mg; iodine, 0.65 mg; copper, 80 mg; selenium, 0.35 mg; retinyl acetate, 0.008 MIU; cholecalciferel, 0.002 MIU; vitamin E, 0.009 MIU; vitamin K, 0.6mg; thiamin, 1.0mg; riboflavin, 4.5 mg; niacin, 56 mg; pantothenic acid, 8.2 mg; pyridoxine, 1.9 mg; biotin, 0.05 mg; folic acid, 0.6 mg; vitamin B_{12}, 0.009 mg; choline chloride, 400 mg.

Sampling and analysis

Exp. 1: The blood samples were taken from wing vein at the time of 2 h and 24 h after heat exposure from each experimental bird. The plasma was separated at 3,000 rpm after blood samples were thoroughly mixed with heparin (20 IU/ml), and stored at -20℃ until analysed. The following parameters were determined with progressive determining method: AKP and ACP, lactic dehydrogenase (LD) and creatine kinase (CK). Concentrations of plasma glucose (G) and calcium (CA) were determined with terminal method and colorimetry method, respectively. The determina-

tion instrument was Monarch-1000 auto-biochemical analyser.

Exp. 2 and 3: The blood was sampled at the end of 24-h heat exposure from each bird of every experimental group. The plasma levels of LD, CK, glucose, K+, CL-, uric acid (UA), and urea-nitrogen (UN) were determined in both experiments. The UA and UN were determined by urease method with Auto-Biochemistry Analyser (Monarch-100). The concentrations of K and CL were determined with K-Na-Cl Analyser (CIBA-CORNING 644).

Exp. 4: The blood samples were taken from wing vein 24 h after thermal exposure, and the serum was separated and stored at -20℃ until determination. The concentrations of T3, T4, and insulin were determined by radioimmunoassay (RIA) method. The determination reagents were provided by Northern Center of Immunity Reagent (Beijing).

Statistical analysis

Data obtained were analyzed by using variance procedure of SAS package (1989), and treatment means were compared using Duncans multiple range test (Duncan, 1955).

treatments ($p>0.05$) in different exposure time, respectively. However, these indices had significant changes at different exposure time (2, 24 h) ($p<0.01$) (table 2).

The significant effect of thermal environment was observed on the level of LD after 2 or 24 h heat exposure ($p<0.01$), and the effect had a trend to be enforced by prolonged exposure time. The highest LD level was observed in 35℃ and 60% RH treatment group. The levels of plasma glucose and CK were not changed after 2 h heat exposure ($p>0.05$), but changed significantly 24 h later. The glucose concentration and CK level were elevated significantly after 2 h heat exposure ($p<0.01$).

Experiment 2

The results of Experiment 2 showed temperature treatment had significant effects on K, Cl, UA, UN and LD levels ($p<0.01$). The K concentration was increased as the temperature elevated from 10℃ to 30℃, but decreased sharply at high temperature (35℃) ($p<0.01$) and declined by cold exposure (10℃) ($P<0.01$). The Cl level was only decreased by high temperature (35℃) ($p<0.01$), and was not affected by

Table 2. Effects of thermal treatments on various plasma parameters in Experiment 1

Indices[a]	Time exp.	20℃ 60% RH	35℃ 35% RH	35℃ 60% RH	35℃ 85% RH	Means[b]	Sign.
LD	2	406.7 ± 66.4^{ab}	348.7 ± 113.1^{b}	464.5 ± 187.1^{a}	315.6 ± 42.7^{b}	376.4^{X}	2 h treat: $p<0.05$
IU/L	24	311.0 ± 174.2^{B}	264.0 ± 71.5^{B}	519.7 ± 274.6^{A}	301.0 ± 66.5^{B}	348.9^{Y}	24 h treat: $p<0.01$
							Time $p<0.01$
CK	2	1975.7 ± 844.2	1390.6 ± 1222	2336.5 ± 1344	1595.4 ± 848.2	1824.6^{Y}	2 h treat: $p>0.05$
IU/L	24	2272 ± 1107^{b}	2551 ± 1327^{b}	4195 ± 2362^{a}	3519 ± 1430^{ab}	3134.5^{X}	24 h treat: $p<0.05$
							Time $p<0.01$
CA	2	2.73 ± 0.40	3.01 ± 0.44	3.01 ± 0.37	3.10 ± 0.29	2.96^{X}	2 h treat: $p>0.05$
mMol/L	24	2.41 ± 0.37	2.24 ± 0.16	2.29 ± 0.5	2.32 ± 0.33	2.31^{Y}	24 h treat: $p>0.05$
							Time $p<0.01$
ACP	2	28.0 ± 17.2	40.3 ± 22.3	24.6 ± 26.5	33.8 ± 29.3	31.7^{Y}	2 h treat: $p>0.05$
IU/L	24	42.2 ± 25.0	53.3 ± 17.5	46.1 ± 19.7	41.9 ± 18.8	45.8^{X}	24 h treat: $p>0.05$
							Time $p<0.01$
AKP	2	7082.5 ± 4457	5602.0 ± 3559	9457.7 ± 6707	6786.0 ± 3192	7232.1^{X}	2 h treat: $p>0.05$
IU/L	24	6986.0 ± 5302	3375.0 ± 1643	4375.6 ± 3158	4631.3 ± 1844	4842.0^{Y}	24 h treat: $p>0.05$
							Time $p<0.01$
G	2	13.7 ± 1.7	14.4 ± 1.6	14.2 ± 2.0	14.8 ± 1.3	14.3	2 h treat: $p>0.05$
mMol/L	24	12.2 ± 1.4^{B}	13.7 ± 1.2^{Ab}	14.9 ± 1.7^{A}	14.7 ± 0.8^{A}	13.9	24 h treat: $p<0.01$
							Time $p>0.05$

[a,b] Means with different capital letters in the same line or column differed high significantly ($p<0.01$), means with different small letters in the same column differed significantly ($p<0.05$).

RESULTS

Experiment 1

The results showed the concentrations of ACP, AKP and calcium were not affected by different heat

other temperature treatments (10℃ or 30℃). Cold exposure (10℃) could increase plasma UA and UN concentration significantly ($p<0.01$). Temperature treatment had no significant effects on glucose and CK levels in this experiment ($p>0.05$). The significant

effect of humidity treatment was only observed in Cl concentration ($p<0.01$), which was decreased by high relative humidity (85% RH). There were significant interaction between temperature and humidity on UA, glucose, LD and CK levels ($p<0.01$).

Experiment 3

The results of Experiment 3 showed that the LD and CK were affected significantly by temperature ($p<0.01$) and humidity treatment ($p<0.01$), respectively, but the K, Cl, glucose, UN and UA levels were not significantly changed ($p>0.05$) (table 4). The activity of LD and CK was elevated by high temperature or high humidity treatment, respectively. The interaction of temperature and humidity was significant in plasma K ($p<0.01$) and LD ($p<0.05$) levels.

Experiment 4:

The results of Experiment 4 indicated that the serum concentration of T3 was decreased significantly by high exposure temperature ($p<0.05$), but was not affected by humidity treatments ($p>0.05$). Contrary to the changes of T3, the concentration of T4 was not affected by temperature ($p>0.05$), but by humidity ($p<0.01$) which had a trend to be increased by higher or lower humidity (table 5). The significant interaction of temperature and humidity was showed both in T3 and T4 ($p<0.01$). Neither the temperature nor humidity had significant effects on serum insulin level ($p>0.05$), but they have significant interaction ($p<0.01$).

DISCUSSION

Effect of temperature and RH on plasma ACP and AKP levels

No significant effects of temperature or humidity on activities of ACP and AKP were observed after 2 or 24 h heat exposure in Experiment 1, which. was in agreement with the report of Bogin (1981), who could not find any significant change in AKP in his acute heat exposure experiment (40℃, 2 h). However, there are several different reports. Vysotskaya et al. (1979) reported that the ACP activity reach the tiptop one hour after 40℃-heat-exposure. Ostrowski-Meissner (1981) noted that the AKP level decreased by 15 min of 42℃-heat-treatment. The reason might relate to the temperature of exposure, variety of birds and the level of basal metabolism of experimental birds that were concerned with temperature history, nutrition level, etc. The results indicated that the activities of ACP or AKP in heat-stressed birds were inconsistent and needed to be studied further.

Effect of temperature and RH on plasma calcium concentration

McCormick and Garlich (1982) reported that the plasma calcium level was decreased by heat stress, but this result was not confirmed by our experiment, which showed that the calcium concentration was not affected by heat treatment but different exposing time. The possible reasons for different results in the reports might be the degree of temperature elevated, exposure time and determining time. The results suggested the conclusion would be variable by different conditions.

Effect of temperature and RH on plasma glucose level

Exposure to hot environment might result in heat stress. The secretion of glucocorticoid was increased in the heat-stressed birds. That might cause the degradation of glycogen, hydrolysis of lipid and gluconeogenesis of amino acids, and led to high plasma concentration of glucose, free fatty acids and others in turn (stress physiology). Our results of three experiments showed that the effect of temperature or humidity on plasma glucose concentration was inconsistent. In Experiment 1, the plasma glucose concentration was not affected by 2 h heat exposure ($p>0.05$), but by 24 h heat exposure. This result confirmed the conclusion of Ostrowski-Meissner (1981) that the plasma glucose concentration was elevated by high temperature. However, this effect was not demonstrated in Experiment 2 and 3, though it had a trend to be elevated by high temperature. Bogin (1981) and Braganza et al. (1973) did not find a significant change in plasma glucose level in their experiments. The different degrees of heat stress might be the reason for different response of glucose level to thermal environment.

In Experiment 1, the RH appeared to exert a significant effect on plasma glucose concentration. The glucose concentration was increased by high RH (60-85%) under high temperature. On consideration of the effect of high RH (60-85%) on glucose concentration, it might indicate that the adverse effect of high temperature might be aggravated by medium and high RH. However, this effect of humidity was not observed in Experiment 2 and 3, though there was a significant interaction between temperature and RH ($p<0.01$) in Experiment 2.

Effect of temperature and RH on LD and CK levels

It was reported that the activities of LD and CK were elevated in hot environment (Ostrowki-Meissner, 1981; Sharma and Gangwar, 1986; Hocking et al., 1994). The similar result was also observed in experiment one, but the result was not consistent in different treatments and various determining times. The significant effect of heat treatment on LD was found at 35℃ and 60% RH treatment, and this effect became more significant along with the exposure time

prolonged ($p<0.05$ at 2 h, $p<0.01$ at 24 h). The effect of temperature on LD and CK activity was confirmed by Experiment 3, but was not by Experiment 2. In Experiment 2, the LD activity was increased by cold exposure, but was unaffected by high-temperature exposure. Though the CK level had a trend to be increased by the rising of temperature, no significant changes were observed ($p>0.05$). The results of Experiment 3 confirmed the conclusion of the first experiment that LD and CK activity was raised by high temperature (33℃) and high humidity. As the experimental temperatures and the age of experimental birds were different in the three experiments, so the different temperatures or different thermal requirement of birds at different week of age (3 or 7 wk) might be the reason of different responses in LD and CK levels.

In Experiment 1, we observed an interesting phenomenon that the significant effect of high temperature on LD and CK was observed only in medium humidity (60% RH), but was not in high nor low humidity (85% or 35% RH). This result suggested that the adverse effect of high or low humidity under high temperature environment. But the results of Experiments 2 and 3 showed high humidity (85% RH) had significant effect when compared to low humidity (35% RH). Thus the effect of humidity under high temperature needed to be evaluated further. Furthermore, the effect of humidity seemed to be going along with the effect of temperature according to the results of Experiments 2 and 3.

The result of this experiment indicated that LD was a more sensitive index to reflect the effect of heat stress than ACP or AKP, as was reported by Sharma and Gangwar (1986).

These results suggested that broilers at different growth periods might have different thermal requirements and would response differently to heat exposure.

The effect of temperature and RH on K and Cl

The result of Experiment 2 showed that, compared to 25℃ treatment, the plasma K level was increased by 30℃ temperature treatment, but decreased dramatically at 33℃ and 10℃ treatments. However, this result was not significant in Experiment 3. In the previous studies, many researchers had reported the declining of plasma K in birds under heat stress (Smith and Teeter, 1987; Deyhim and Teeter, 1991). Belay and Teeter (1993) reported that the plasma K concentration was decreased by heat stress. But the results of our experiment indicated that the influence of heat exposure on plasma K level might be dependent on the degree of heat stress and had some uncertainty in response to heat stress. Unlike its response to temperature, plasma K concentration was not changed by humidity in both two experiments.

The effects of temperature and humidity on plasma Cl content were also different in two experiments. The result showed that the Cl level was decreased significantly by high temperature (33℃) in Experiment 2, but was unaffected in Experiment 3. Belay et al. (1992) reported that the urine Cl excretion was increased. As the urine Cl excretion increased, the plasma Cl would be elevated. Belay and Teeter (1993) demonstrated this conjecture in their following study and reported that plasma Cl level was increased by high temperature. The results of our experiment, however, could not confirm the above reports.

The results in the two experiments showed plasma K and Cl concentration had similar reaction to temperature or humidity.

The effect of temperature and humidity on UN and UA

The UA and UN concentration were increased by low temperature in Experiment 2. That result confirmed the report of Ostrowski-Meissner (1981), who indicated that plasma uric acid level was increased by low-temperature stress (10℃). However, this conclusion was not supported by the result of Experiment 3. As the age of experimental birds were different, the cold resistance at different growth period might be the reason.

The effect of temperature and RH on T3

As we know that thyroid has important effect on metabolism. Freeman (1970) approved that the ability of neonatal chicks maintaining body temperature at 20℃ by injection of triiodothyronine or thyronine. However, the adjustment of thyroid to the change of thermal environment might be a slow process. Stahl et al. (1961) reported that the secretion rate of thyroid increased continuously at the 190 d when experimental birds were exposed to 4.5℃.

In the long-term heat exposure experiment, the T3 level was decreased (Williamson et al., 1985; Cogburn and Freeman, 1987). However, there were different reports about the effect of acute heat stress on T3 concentration. Rudas and Pethes (1984) found that T4 level was decreased at the first hour after the experimental cocks, which were adapted to 23℃, were exposed to 10 or 35℃, but T3 level had no significant change. Bobek et al. (1980) reported that T3 level was increased firstly and restored to basal level at 10 h and increased continuously to 50 h after heat exposure, but meanwhile the T4 level went on decreasing, when experimental Japanese quails were exposed from 22℃ to 34-35℃. The result of our study showed that T3 level was decreased significantly and T4 level was not changed by 24 h heat exposure. One reason might be that the birds made a rapid

adjustment in secretion rate of thyroid. Another reason might be related to the deiodination of T4 to T3 in liver and kidney tissues, a reaction catalyzed by 5-deiodinase. Rudas and Pethes (1986) reported that cold exposure could improve the transformation of T4 to T3 in the peripheral tissue.

The effect of temperature on T3 seemed to be affected by humidity. Although the RH had no significant effect on T3, it had significant interaction with temperature on T3 levels. When the RH was below 75%, a significantly lower concentration of T3 was observed in higher temperature treatment. However, this effect was not seen when humidity was more than 75% RH. The RH treatment had significant effect on T4 ($p<0.01$), the medium RH (60% RH) group was lower than other RH treatments. The reason needed to be investigated further.

The time response of plasma indices

The plasma indices reflected the changes of metabolism or the state of organs or tissues. In the study on heat stress, the plasma indices was used as the evidences of stress. In the past study, the exposure time was uncertain and changed from several minutes to many hours. The different exposure time or taking time of blood samples might reflect various stages of heat stress. The result of Experiment 1 showed that different sampling time of blood had significant effect ($p<0.01$) on all indices, except that of glucose ($p>0.05$), in all experimental treatments and even the control group. No significant effect of temperature or humidity was considered, the similar in-phase responses of ACP, AKP and calcium in control and experimental groups were believed to be the effect of sampling time. This observation suggested that plasma indices themselves might have a wide variation. As the blood biochemical indices were commonly used to reflect the effect of heat stress, the best blood sampling time should be evaluated further.

The sensitivity and variation of biochemical indices

Meltzer (1982, 1983) indicated that the sensitivity

Table 3. Effects of thermal treatments on plasma parameters in Experiment 2

RH (%)	Temperature (℃) 10	25	30	33	Means[b]	Sign
			K+, mMol/L			
35	5.02±0.46	5.39±0.41	5.91±0.45	5.07±0.49	5.35	Temp: $p<0.01$
85	5.01±0.34	5.51±0.32	5.92±0.94	4.66±0.27	5.28	RH: $p>0.05$
Means[a]	5.01^{C}	5.45^{B}	5.92^{A}	4.86^{C}		Temp×RH: $p>0.05$
			CL-, mMol/L			
35	120.3±4.4	117.8±6.5	122.6±8.6	112.0±3.7	118.2^{X}	Temp: $p<0.01$
85	115.2±5.7	117.2±8.4	118.8±4.2	107.0±4.6	114.6^{Y}	RH: $p<0.01$
Means[a]	117.8^{A}	117.5^{A}	120.7^{A}	109.5^{B}		Temp×RH: $p>0.05$
			UA, mMol/L			
35	302.8±90.5	291.3±111.2	262.6±43.0	224.2±47.7	270.2	Temp: $p<0.01$
85	491.6±162.3	235.1±58.3	259.5±90.3	224.2±43.7	302.6	RH: $p>0.05$
Means[a]	397.2^{A}	263.2^{B}	261.1^{B}	224.2^{B}		Temp×RH: $p<0.01$
			UN, mMol/L			
35	0.84±0.35	0.63±0.16	0.70±0.19	0.43±0.14	0.65	Temp: $p<0.01$
85	0.78±0.31	0.47±0.18	0.57±0.30	0.56±0.14	0.59	RH: $p>0.05$
Means[a]	0.81^{A}	0.55^{B}	0.64^{AB}	0.49^{B}		Temp×RH: $p>0.05$
			G, mMol/L			
35	13.30±1.31	14.03±1.37	14.87±1.00	14.34±0.87	14.13	Temp: $p>0.05$
85	14.56±1.11	14.91±0.78	13.86±1.51	15.19±0.90	14.63	RH: $p>0.05$
Means[a]	13.93	14.47	14.37	14.76		Temp×RH: $p<0.01$
			LD, IU/L			
35	170.5±47.0	319.1±63.7	272.8±40.5	224.3±39.9	246.7	Temp: $p<0.01$
85	218.9±45.0	223.5±49.8	267.2±88.3	294.2±92.3	251.0	RH: $p>0.05$
Means[a]	194.7^{B}	271.3^{A}	270.0^{A}	259.3^{A}		Temp×RH: $p<0.01$
			CK, IU/L			
35	861.8±463.5	1636.5±1211	1423.7±774.8	964.1±381.5	1139.7	Temp: $p>0.05$
85	1499.5±1012	747.9±338.5	1784.4±2135	3211.0±3164.3	1810.7	RH: $p>0.05$
Means[a]	1180.7	893.6	1604.1	2087.6		Temp×RH: $p<0.01$

[a,b] The means with different superscripts in the same line or column differ significantly ($p<0.01$).

Table 4. Effects of thermal treatments on plasma parameters in Experiment 3

RH (%)	Temperature (℃)				Means[b]	Sign
	7	24	28	32		
			K+, mMol/L			
35	4.13±0.35	4.71±0.32	4.50±0.43	3.92±0.57	4.31	Temp: p>0.05
85	4.60±0.32	4.25±0.52	4.75±0.55	4.86±0.74	4.61	RH: p>0.05
Means[a]	4.36	4.48	4.63	4.39		Temp×RH: p<0.01
			CL-, mMol/L			
35	112.9±5.2	112.3±5.8	109.3±2.8	106.6±4.7	110.3	Temp: p>0.05
85	112.0±4.4	110.9±7.8	108.4±6.4	111.8±3.8	110.8	RH: p>0.05
Means[a]	112.4	111.6	108.8	109.2		Temp×RH: p>0.05
			UA, mMol/L			
35	169.7±64.5	163.8±52.5	177.4±81.8	148.8±61.4	164.9	Temp: p>0.05
85	158.5±64.8	186.4±106.8	167.3±49.1	178.8±54.5	172.7	RH: p>0.05
Means[a]	164.1	175.1	172.3	163.8		Temp×RH: p>0.05
			UN, mMol/L			
35	0.45±0.22	0.65±0.35	0.33±0.18	0.54±0.16	0.49	Temp: p>0.05
85	0.44±0.18	0.49±0.20	0.59±0.16	0.51±0.21	0.51	RH: p>0.05
Means[a]	0.44	0.57	0.46	0.53		Temp×RH: p>0.05
			G, Mmol/L			
35	13.21±1.41	12.78±1.44	14.83±0.89	14.48±1.43	13.82	Temp: p>0.05
85	13.74±1.50	13.83±1.15	13.50±1.53	14.24±1.56	13.83	RH: p>0.05
Means[a]	13.48	13.30	14.16	14.36		Temp×RH: p>0.05
			LD, IU/L			
35	184.1±34.9	273.9±48.5	303.4±79.5	293.4±63.8	263.7^{Y}	Temp: p<0.01
85	253.5±86.3	250.3±107.0	416.6±159.0	474.4±160.6	348.7^{X}	RH: p<0.01
Means[a]	218.8^{B}	262.1^{B}	360.0^{A}	383.9^{A}		Temp×RH: p<0.05
			UK, IU/L			
35	1460.3±643.1	1691.9±296.1	2341.3±899.7	2508.5±1442.1	2000.5^{Y}	Temp: p<0.01
85	1575.1±695.0	2050.8±837.9	3266.3±1232.2	3314.4±1441.8	2551.6^{X}	RH: p<0.05
Means[a]	1517.7^{C}	1871.3^{bC}	2803.8^{AB}	2911.4^{A}		Temp×RH: p>0.05

[a,b] The means with different superscripts in the same line or column differ significantly (p<0.01).

of broilers to high and low temperature changed along with the growth, and the broilers became more susceptible to high temperature and less susceptible to low temperature. As humidity had some influence on the effect of temperature (Chwalibog and Eggum, 1989), the exact requirement of temperature and humidity of broilers in different growing periods was still debatable.

The results of our study indicated the same indices might have different responses to heat treatment in different experiments. One reason might be the different requirement to temperature and humidity in different growth period. Another reason might be the variations of the biochemical indices themselves.

The variations of biochemical indices appeared in two sides. One side was that the biochemical indices could not keep the consistent responses to the effects of temperature and humidity in the four experiments. For example, plasma LD levels, considered as a more sensitive index to reflect the effect of stress (Sharma and Gangwar, 1986), had different degrees of response at 2 or 24 h of determining time in Experiment 1 (table 2), and different reaction to temperature or humidity in Experiment 2 and 3 (table 3, 4). Other indices had similar responses to the effect of temperature or humidity. Another side was that the determined biochemical indices had big variation in different experiments (table 6). Among the indices determined, the biggest variation was observed in CK values (40.46-69.10%) and the smallest one was Cl values (4.61-4.93%). It suggested that the big variation of biochemical indices themselves might cover the response of indices to temperature and humidity treatments.

CONCLUSION

It was concluded that the different sampling time of blood had significant effect on the results. Broilers at different growth periods might have different thermal requirements and would response differently to heat exposure. The different indices had different

Table 5. Effects of thermal treatments on serum hormone level of heat-stressed birds

RH (%)	Temperature (℃)				Means[b]	Sign
	24	27	30	33		
			T3, ng/ml			
30	0.558 ± 0.296^{ab}	0.955 ± 0.470^{a}	0.569 ± 0.426^{ab}	0.471 ± 0.365^{b}	0.638	Temp: $p<0.05$
45	0.724 ± 0.497^{aB}	0.482 ± 0.488^{B}	1.106 ± 0.862^{A}	0.254 ± 0.180^{B}	0.642	RH: $p>0.05$
60	0.929 ± 0.611^{a}	0.602 ± 0.434^{ab}	0.378 ± 0.401^{b}	0.467 ± 0.288^{b}	0.594	Temp×RH: $p<0.01$
75	0.654 ± 0.419^{a}	0.651 ± 0.255^{a}	0.499 ± 0.206^{a}	0.522 ± 0.379^{a}	0.582	
90	0.611 ± 0.525^{a}	0.699 ± 0.452^{a}	0.568 ± 0.394^{a}	0.738 ± 0.378^{a}	0.654	
Means[a]	0.695^{a}	0.678^{a}	0.624^{ab}	0.490^{b}		
			T4, ng/ml			
30	24.05 ± 6.44^{ab}	14.04 ± 11.15^{bc}	17.75 ± 6.44^{abc}	24.08 ± 15.74^{a}	19.98^{Xx}	Temp: $p>0.05$
45	15.81 ± 13.46^{b}	24.08 ± 15.56^{a}	23.88 ± 8.05^{a}	23.75 ± 5.61^{a}	21.88^{X}	RH: $p<0.01$
60	16.73 ± 7.14^{b}	13.67 ± 4.32^{c}	12.62 ± 4.36^{c}	13.40 ± 8.74^{b}	14.11^{Y}	Temp×RH: $p<0.01$
75	15.67 ± 7.06^{b}	23.33 ± 6.84^{ab}	22.85 ± 12.92^{ab}	24.68 ± 15.47^{a}	21.63^{X}	
90	26.58 ± 10.3^{a}	14.04 ± 5.23^{bc}	13.83 ± 9.46^{bc}	13.83 ± 8.38^{b}	17.07^{xY}	
Means[a]	19.77	17.83	18.19	19.95		
			Insulin, μIU/ml			
30	8.22 ± 5.57^{AB}	5.26 ± 2.34^{AB}	5.17 ± 2.81^{B}	9.38 ± 2.97^{A}	7.01	Temp: $p>0.05$
45	6.90 ± 3.32^{AB}	6.71 ± 3.03^{AB}	9.28 ± 6.44^{A}	4.84 ± 2.87^{B}	6.93	RH: $p>0.05$
60	5.69 ± 2.66^{b}	6.33 ± 2.48^{ab}	9.32 ± 3.83^{a}	8.98 ± 2.49^{a}	7.58	Temp×RH: $p<0.01$
75	4.39 ± 1.99^{b}	6.81 ± 2.68^{ab}	5.55 ± 3.67^{ab}	8.02 ± 1.68^{a}	6.19	
90	8.39 ± 3.02^{a}	5.69 ± 2.87^{ab}	4.83 ± 2.69^{b}	6.51 ± 3.96^{ab}	6.35	
Means[a]	6.72	6.16	6.83	6.95		

[a,b] Means in the same column with different small letters differed significantly ($p<0.05$), means in the same column or line with different capital letters differed high significantly ($p<0.01$).

Table 6. The coefficient of variation of various plasma parameters in Experiment 1, 2, and 3

Indices	Exp. 1	Exp. 2	Exp. 3
LD, IU/L	39.51	23.42	29.19
CK, IU/L	49.44	69.10	40.46
G, mMol/L	9.27	7.76	9.92
K, mMol/L	--	8.54	10.67
Cl, mMol/L	--	4.93	4.61
UA, mMol/L	--	27.22	39.43
UN, mMol/L	--	35.30	42.12

responses to temperature and humidity treatments and appeared big variation in different experiments. The big variation of biochemical indices themselves might cover the response of indices to temperature and humidity treatments.

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