



**Full Length Article**

## Effects of Extreme Heat Stress on Growth Performance, Lymphoid Organ, IgG and Cecum Microflora of Broiler Chickens

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### Abstract

In this study, the effect of extreme heat diet on growth performance, lymphoid organ, blood immunoglobulin and cecum microflora change in broilers exposed to continuous lighting and extreme heat stress (EHS) was studied. Broilers raised under normal environment temperature (25°C) or extreme heat stress temperature (33±2°C), and consumed chow diet (CD) or extreme heat stress diet (EHSD). Five hundred Ross 308 days-old commercial broilers were arranged in a completely randomized block design of 5 treatment groups with 4 repetitions (25 heads per repetition pen). The broilers were divided into: T1 (normal environment+CD); T2 (EHS+CD); T3 (EHS+EHSD in which the tallow in CD was substituted by soy oil and contained 5% molasses); T4 (EHS+EHSD in which the tallow in CD was substituted by soy oil and contained 5% molasses, and 1.5 times more methionine and lysine than CD), and T5 (EHS+EHSD in which the tallow in CD was substituted by soy oil, contained 5% molasses, 1.5 times more methionine and lysine than CD, and 300ppm of vitamin C). The EHS significantly reduced the body weight gain and feed intake. The blood immunoglobulin, bursa of Fabricius, thymus, and spleen weight were significantly reduced when broilers were exposed to EHS. Compared to the normal environment temperature group, the cecum *Lactobacillus* sp. was low in the EHS treatment group, while *Escherichia* sp., *Salmonella* sp. and total aerobic bacteria in the EHS treatment group were high. A statistically significant difference was acknowledged between the treatment groups. © 2013 Friends Science Publishers

**Keywords:** Extreme heat stress; Immunoglobulin; Lymphoid organ, Cecum microflora

### Introduction

Extreme heat stress during the summer is of great interest in the poultry industry. When broilers are exposed to extreme heat stress, their feed intake is reduced and a rapid drop in growth rate as well as increase in mortality can cause an added economic loss to poultry farms. When broilers are exposed to 32°C, their feed intake is reduced by 24% and a high temperature environment can have an adverse effect on the specific immune reaction of broilers (Niu *et al.*, 2009). Since broiler chickens don't have sweat glands and are covered with a feather, they don't have the biological characteristics to enable them to release heat from their body surface. When broilers are exposed to extreme heat become extreme heat stressed, they demonstrate panting along with open mouth respiration to maintain homeostasis. Due to the rapid body temperature increase, this can ultimately cause mortality (Austic and Nesheim, 1990; Han *et al.*, 2010). The body temperature of an adult fowl is 41–42°C, however the thermal comfort environment temperature is known to be about 25°C (Cooper and

Washburn, 1998). When the environment temperature increases, body temperature increases and in general, heat stress appears at over 30°C (Donkoh, 1989). Between body weight and a resistance to heat stress in a growing broiler, a negative phenotype correlation was reported (Washburn *et al.*, 1980). There are a number of reports that reduced feed intake and growth rate suppression in broilers raised in a high temperature environment are related to broilers' gene by breed, age, feed utilization, and body weight gain (Deaton *et al.*, 1972; Suk and Washburn, 1995; Quinteiro-Filho *et al.*, 2001). When the protein level increases under the heat stress, even more metabolic heat is produced, thus it is helpful to increase essential amino acids like methionine and lysine. As well, stimulating feed intake by providing high preference ingredients such as soy oil or molasses, and providing vitamin C are helpful to minimize heat stress (Leeson and Summers, 1991). While various studies on heat stress in broilers have been made, little has been known about an extreme heat stress diet using the various nutrient ingredients suggested above.

The goal of this study was to assess the effects of an

extreme heat stress diet with soy oil and extreme heat stress on the lymphoid organ weight, levels of IgG, cecum microflora and growth performance of broiler chickens.

## Materials and Methods

### Experimental Design and Birds

All experiment procedures that included animals followed the scientific and ethical regulations proposed by the European Animal Experiment Handling License Textbook (Scot PIL Training Manual, 1994) and board approval was attained from the Animal Experiment Ethics Committee in Kangwon University, Republic of Korea. Five hundred day-old Ross 308 broiler chicks were arranged in a completely random design of 5 treatment groups on the day of hatching. Each group had 4 repetitions, and each repetition contained 25 broilers. The treatment groups were divided into: T1 (normal environment + chow diet (CD)); T2 (extreme heat stress, (EHS)+CD); T3 (EHS + extreme heat stress diet (EHSD) in which the tallow in CD was substituted by soy oil and contained 5% of molasses); T4 (EHS+EHSD in which the tallow in the CD was substituted by soy oil and contained 5% of molasses, 1.5 times more methionine and lysine than normal feed); and T5 (EHS+EHSD in which the tallow in CD was substituted by soy oil and contained 5% of molasses, 1.5 times more methionine and lysine than CD, and vitamin C 300 ppm). The CD was mixed in to satisfy the nutrient requirement, mainly with a corn and soybean meal based on the NRC (1994) feeding standard (Table 1). T3-T5 EHSD were mixed by substituting the tallow routinely added in CD with soy oil with high energy utilization, adding molasses known to have high preference ability, and increasing methionine, lysine, and vitamin C higher than CD. During the entire experimental period, 24 hour continuous lighting was given to the chicks and the chicks were raised under the standard condition were given free access to drinking water (25-28°C). In each pen, a rice husk was laid as a 10 cm thick bed. The temperature of the pen was maintained at 33°C from the day of entrance for 3 days and decreased by 2~3°C every week, and from the day 22, it was maintained at normal environment temperature (25°C). From day 28 to day 32, EHS (33±2°C) and relative humidity of 70% were maintained for 5 hours a day (11:00-16:00).

### Slaughtering and Blood Collection

Experiment diet was removed for 12 hours before slaughtering and when the experiment was completed, 20 chicks (5 chicks from each pen) of which the body weight is close to average were selected from the repetition pen of each treatment group for blood collection. They were stably sacrificed without stress by cervical dislocation according to recommendation for experiment animal euthanasia (Close *et al.*, 1997). A total of 3 mL of blood was collected from the heart using a plain tube (Greiner Co Ltd, Australia) and the

blood was allowed to coagulate for 30 min. at room temperature. It was centrifuged for 10 min. at 3,000 rpm to separate the serum, rapidly frozen in liquid nitrogen at -196°C, then stored at -20°C until the next biochemical analysis.

### Determination of Serum Immunoglobulin

Serum immunoglobulin was measured using a chicken IgG ELISA kit (Bethyl Laboratories, Montgomery, TX, USA) following the manufacturer's protocol. The level of immunoglobulin was calculated by measurement of the absorbance by precision of a microplate reader (Molecular Devices Inc, New York, USA) at 450 nm.

### Cecum Microflora

Immediately after slaughtering, the cecum was removed by the anaerobic method and maintained in an ice box to study the cecum microflora. The cecum was maintained in an anaerobic condition in sealed anaerobic jars (Oxoid, Basingstoke, UK) and equipped with AnaeroGen sachets (Oxoid, Hampshire, UK). The contents of the cecum was blended with sterilized anaerobic saline solution (phosphorus buffered saline; PBS 0.1 M, pH 7.0) and diluted 10 times (1:9, wt/vol), then serial dilution was continued. All procedures were conducted in an anaerobic condition in an anaerobic chamber (5% hydrogen, 5% CO<sub>2</sub>, balanced nitrogen). To culture, 100 µL each was divided from diluted 10<sup>-2</sup>~10<sup>-7</sup> and a culture was performed in a sterilized flat selective medium, that is *Escherichia* sp. (McConkey purple agar), *Lactobacillus* sp. (MRS agar, Oxoid, Basingstoke, UK), *Salmonella* sp. (SS agar, Difco), and total aerobic bacteria (Nutrient agar, Difco). *Escherichia coli* sp., *Salmonella* sp., and the total aerobic bacteria were cultured aerobically at 37°C for 24 h and *Lactobacillus* sp. was stationary cultured under an anaerobic condition using sealed anaerobic jars equipped with AnaeroGen sachets at 37°C for 48 h, then the colony count was found by microbial counter on each flat medium. A colony count of all microbes was suggested by taking the common logarithm as CFU (colony-forming unit/g of wet of cecum content).

### Statistical Analysis

Data was analyzed by ANOVA using GLM procedure by SAS software's GLM procedure, and the statistical significant difference of all data was tested at P<0.05 by Duncan's multiple range test (SAS, 2004).

### Results

The growth performance of the broilers during the entire period is shown in the Table 2. Body weight gain and feed intake were the highest in the T1 result, and it was significantly high in a decreasing order of T2, T3, T4 and T5, but there was no statistically significant

**Table 1:** Composition of chow diets for broiler chickens

Ingredients (% as-fed)	Growing stage	
	Starter (0-21 days)	Grower (22-32 days)
Yellow corn	52.00	50.00
Soybean meal	34.00	25.00
Corn gluten meal	4.70	5.70
Wheat meal	-	10.00
Tallow	5.00	5.00
Limestone	1.25	1.25
Dicalcium phosphate	1.70	1.70
Sodium chloride	0.25	0.25
DL-methionine (50%)	0.30	0.30
L-lysine HCl (78%)	0.30	0.30
Trace mineral premix <sup>1)</sup>	0.34	0.34
Vitamin premix <sup>2)</sup>	0.16	0.16
Total	100	100
Chemical composition		
ME (kcal/kg)	3,100	3,150
Crude protein (%)	22.00	20.00
Lysine (%)	1.32	1.15
Methionine (%)	0.52	0.50
Methionine+Cystine (%)	0.78	0.73
Calcium (%)	1.00	0.90
Available phosphorus (%)	0.45	0.40

<sup>1)</sup>Supplied per kilogram of diet: Fe, 80 mg; Zn, 80 mg; Mn, 70 mg; Cu, 7 mg; I, 1.20 mg; Se, 0.30 mg; Co, 0.70 mg. <sup>2)</sup>Supplied per kilogram of diet: vitamin A (retinyl acetate), 10,500 IU; vitamin D<sub>3</sub>, 4,100 IU; vitamin E (DL- $\alpha$ -tocopheryl acetate), 45 mg; vitamin K<sub>3</sub>, 3.0 mg; thiamin, 2.5 mg; riboflavin, 5 mg; vitamin B<sub>6</sub>, 5 mg; vitamin B<sub>12</sub>, 0.02 mg; biotin, 0.18 mg; niacin, 44 mg; pantothenic acid, 17 mg; folic acid, 1.5 mg

**Table 2:** Growth performance of broiler chickens fed experimental diets for 32 days (g/head)

Item	Groups					Pooled SEM
	T1	T2	T3	T4	T5	
Body weight gain	1,581 <sup>a</sup>	1,471 <sup>b</sup>	1,401 <sup>c</sup>	1,257 <sup>d</sup>	1,235 <sup>d</sup>	18.770
Feed intake	2,624 <sup>a</sup>	2,404 <sup>b</sup>	2,228 <sup>c</sup>	2,010 <sup>d</sup>	2,064 <sup>d</sup>	24.571
Feed efficiency	0.60 <sup>c</sup>	0.61 <sup>b</sup>	0.62 <sup>a</sup>	0.62 <sup>a</sup>	0.60 <sup>c</sup>	0.0022

T1, normal environment+chow diet, CD; T2, extreme heat stress, EHS+CD; T3, EHS+extreme heat diet, EHD in which the tallow in CD was substituted by soy oil and containing 5% molasses; T4, EHS+EHD in which the tallow in CD was substituted by soy oil and containing 5% of molasses, and 1.5 times more methionine and lysine than CD; T5, EHS+EHD in which the tallow in CD was substituted by soy oil, containing 5% of molasses, 1.5 times more methionine and lysine than CD, and 300ppm of vitamin C. a, b, c, d p<0.05

difference between T4 and T5. The feed efficiency ratio was high in a decreasing order of T3, T4, T2, T1 and T5, but there were no statistical significant differences between T3 and T4, and T1 and T5. The result shows that an extreme heat diet cannot enhance the growth performance of broilers exposed to continuous lighting and extreme heat stress.

The serum IgG, immune system lymphoid weight, bursa of Fabricius, thymus, and spleen weight are shown in the Table 3. The serum IgG and bursa of Fabricius, thymus, and spleen weight were significantly highest in T1, compared to T2, T3, T4 and T5, and there were statistically significant differences between each treatment group. Consequently, it was observed that the immune system weight and IgG decreased in broilers exposed to continuous lighting and extreme heat stress.

The cecum microflora change is shown in the Table 4. Probiotics and *Lactobacillus* sp. were highest in T1, compared with T2, T3, T4, and T5, and there were statistically significant differences between each treatment group. Harmful bacteria, *Escherichia* sp., *Salmonella* sp. and total aerobic bacteria were significantly lowest in T1, compared to T2, T3, T4 and T5, and the statistically significant difference was accepted between each treatment group. The result shows that the maintenance of cecum microflora is suppressed when broilers were exposed to continuous lighting and extreme heat stress.

## Discussion

This result shows that body weight and feed intake are significantly affected by continuous lighting and extreme heat stress which is similar to the results of Guo *et al.* (2003). When the broilers are exposed to continuous lighting and extreme heat stress, the growth performance of the broilers significantly dropped. This result aligned with the general trend observed in the heat stress broilers. At atmospheric temperature higher than 20°C, an increase by 10°C, showed a 17% of reduction of feed intake (Austic, 1985), and when broilers were exposed to 32°C, the body weight was reduced by 14% at the age of 2-4 weeks, and by 24% at the age of 4-6 weeks (Geraert *et al.*, 1996). Compared to the treatment group that was exposed to continuous lighting and extreme heat stress (T2) or the groups (T3-5) in which the extreme heat diet was provided during periods of extreme heat stress, the body weight gain of the broilers in T1, raised with normal diet and normal environment, was higher and this is because in T1, the growth of beneficial *Lactobacillus* was facilitated while that the growth of harmful bacteria was suppressed, intestinal microflora was maintained (Table 4), and the animal's health had improved as the serum immunoglobulin increased (Table 3) by the weight increase of lymphoid organs of thymus, spleen and bursa of Fabricius and feed intake was stimulated. Under heat stress, weight gain, feed intake and the feed utilization of broilers were related to body temperature and when they are exposed to the heat stress at 32°C, body weight gain and feed conversion ratio of broilers decreased due to the rapid body temperature increase (Cooper and Washburn, 1998). Also, there was no extreme heat stress diet on extreme heat stress that was found, and it is considered to be attributed by normal drinking water actually supplied in a poultry farm and continuous lighting. When the broilers were exposed to extreme heat stress, continuous lighting and by normal drinking water supply this increased the environment temperature and body temperature and could be an additional cause of extreme heat stress (Deaton *et al.*, 1981; Apeldoorn *et al.*, 1999; Campo *et al.*, 2007).

In the results of exposure to extreme heat stress along with the continuous lighting, it was observed that serum IgG, lymphoid organs, bursa of Fabricius, thymus and

**Table 3:** Serum immunoglobulin and lymphoid organ weight of broiler chickens fed experimental diets for 32 days (Organ weight/body weight, %)

Item	Groups					Pooled SEM
	T1	T2	T3	T4	T5	
Total IgG ( $\mu\text{g/mL}$ )	174.81 <sup>a</sup>	102.58 <sup>b</sup>	84.01 <sup>c</sup>	77.57 <sup>d</sup>	77.85 <sup>d</sup>	0.7019
Bursa of fabricius (%)	0.23 <sup>a</sup>	0.20 <sup>b</sup>	0.19 <sup>b</sup>	0.11 <sup>c</sup>	0.14 <sup>d</sup>	0.0081
Spleen (%)	0.17 <sup>a</sup>	0.15 <sup>b</sup>	0.12 <sup>b</sup>	0.08 <sup>c</sup>	0.09 <sup>c</sup>	0.0020
Thymus (%)	0.22 <sup>a</sup>	0.18 <sup>b</sup>	0.19 <sup>b</sup>	0.14 <sup>c</sup>	0.12 <sup>d</sup>	0.0095

**Table 4:** Cecum microflora in broiler chickens fed experimental diets for 32 days ( $\log_{10}$  cfu/g)

Item	Groups					Pooled SEM
	T1	T2	T3	T4	T5	
<i>Escherichia</i>	6.01 <sup>d</sup>	6.81 <sup>c</sup>	7.10 <sup>b</sup>	9.87 <sup>a</sup>	9.02 <sup>a</sup>	0.2760
<i>Lactobacillus</i>	6.72 <sup>a</sup>	6.50 <sup>b</sup>	5.23 <sup>c</sup>	3.51 <sup>d</sup>	3.38 <sup>d</sup>	0.1815
<i>Salmonella</i>	3.02 <sup>d</sup>	3.29 <sup>c</sup>	3.20 <sup>c</sup>	4.70 <sup>b</sup>	5.12 <sup>a</sup>	0.1318
Total aerobic bacteria	4.17 <sup>c</sup>	5.42 <sup>b</sup>	5.39 <sup>b</sup>	5.78 <sup>a</sup>	5.71 <sup>a</sup>	0.3372

spleen weight significantly decreased. This result conforms to the precedent report that feed intake is reduced by heat stress and this causes insufficient nutrition supply required in the growth of immune system (Bartlett and Smith, 2003; Singh *et al.*, 2006; Han *et al.*, 2010). The increase of IgG in T1 where broilers were raised in the normal environment with chow diet is attributed by the cell proliferation of the immune system (Table 3) and a decrease of IgG in T2-T5 means that humoral immunity was suppressed by extreme heat stress (Park and Park, 2009). The B-cell in bone marrow, IgG, and IgA make Immunoglobulin, and IgM in poultry have similar biological characteristics with mammals. The serum level of IgG is the highest at over 90% and as it is responsible for body immunity, the titer of IgG is the index of humoral immunity (Higgins, 1975). In poultry, the bursa of Fabricius, thymus and spleen are important lymphoid organs in antibody production. The data of the weight increase of thymus in T1 is considered to have increased the proliferating ability of the thymus cell in broilers and it shows that it is able to maintain a constant increase in the production of serum immunoglobulin IgG. In fowls, immunoglobulin is dependent on the mechanism that converts IgM to IgG, or on bursa of Fabricius essential to successful action of IgA, and other related lymph node and intact thymus function (Bienenstock *et al.*, 1973). Therefore, the decreased production of cells containing immunoglobulin and lowered levels of serum immunoglobulin can be considered related to the regression of the lymphoid organs found in broilers under EHS. The growth of the lymphoid organ is the base of the functionality of the immune system. The Bursa of Fabricius is usually constant in poultry and is used for the development of B-lymphocyte and functional maturity study. The thymus and bursa of Fabricius is reduced as the fowl matures and the immune reaction of fowl beyond the point is dependent on spleen and epithelial

lymph node (Wang *et al.*, 2000; Tizard, 2002).

Compared to the treatment group T2, which was exposed to continuous lighting and extreme heat, and T3-T5, exposed to extreme heat stress and had an extreme heat diet intake, the increase of *Lactobacillus*, beneficial to host animals, in T1 is considered to be related to the decrease of harmful *Escherichia*, *Salmonella* and total aerobic bacteria (Table 4). As the cecum microflora of *Lactobacillus* competes with potential pathogens for nutrient and intestinal mucosa attachment sites, it lowers the pathogen group in the cecum, secretes bacteriocin, a material active to *Escherichia*, and generates a substrate to organic acid and other microbes. Most organic acids produced from the fermentation of *Lactobacillus* are lactic acid and acetic acid, and all these substrates can suppress the intestinal clustering by pathogen (Gibson and Wang, 1994; Gibson *et al.*, 1995; Devaraj *et al.*, 2002; Gong *et al.*, 2002; Xu *et al.*, 2002). The reason why the cecum bacteria count of *Escherichia*, *Salmonella*, and total aerobic bacteria significantly decreased in T1 can be regarded as a part of this mechanism. In fowls, the main site of *Salmonella* clustering is the cecum and it is well known that *Salmonella* causes salmonella infection such as diarrhea and serious weight loss. The importance of microbes in the digestive tract is the role of intestinal microbe in synthesis of fermentation product that supplies energy needed in intestinal epithelial cell, stimulation of digestive tract immune system, synthesis of vitamin K, and resistance to clustering of exogenous pathogenic microbe (Shakibaie *et al.*, 2009). In the result of this study, a new finding was verified that extreme heat stress is not beneficial to broiler's cecum microflora or it stimulate proliferation of harmful pathogens *Escherichia*, *Salmonella*, and total aerobic bacteria.

In conclusion, this study suggests that the extreme heat stress diet prepared with soy oil, molasses, methionine, lysine and vitamin C is not at all beneficial to the growth performance of broilers exposed to continuous lighting and extreme heat stress. Therefore, the continuous development of extreme heat stress diet that can cope with the extreme heat stress and additional study on cooling water and light control is considered to be required.

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