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RESEARCH ARTICLE

Effects of heat stress on the immune responses of chickens subjected to thermal manipulation in the pre-hatch period

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ABSTRACT

Heat stress affects the performance of poultry species and also induces immunosuppression. Chickens can be treated by thermal conditioning to have better heat stress tolerance. Our purpose was to determine the effect of acute heat stress on the immune response, i.e. antibody production against Newcastle disease virus (NDV) and change in the proportion of leukocyte components, in chicks subjected to prenatal heat conditioning. Eighty as-hatched broiler chicks from the same parent stock were used: control (40 chicks incubated at 36.7 °C from days 18–20 of embryonic life) and thermally manipulated (TM) (40 chicks incubated at 38.4 °C from day 18–20 of embryonic life; 4 h/day). The chickens were exposed to heat stress: at day 19 (31 °C/8 h) and at day 35 (32 °C/10 h). The first heat stress (day 19) decreased the lymphocyte counts and significantly increased the heterophil counts ($P < 0.05$) in both treatments (from 34.25 to 55% in the controls and from 37 to 60.06% in the TM chicks). The second heat stress (day 35) did not alter the heterophil and lymphocyte profiles of the chickens. Before the heat stress, all of the chickens (control and TM) presented the same positive antibody titres to NDV vaccination. After the first heat stress, 50% of the control samples and 40% of samples from the TM chickens were negative. After the second heat stress, 80% of the TM samples were negative.

KEYWORDS

chicken, incubation, heat stress, Newcastle disease, heterophil, body temperature

INTRODUCTION

High environmental temperature has deleterious effects on the performance of different poultry species and affects the physiological responses of the birds attempting to dissipate heat and maintain body temperature. These adverse effects include changes in respiration rate, plasma ion levels and metabolites (Teeter et al., 1985; Arjona et al., 1990), as well as muscle tissue damage (McKee and Sams, 1997).

Chickens can be physiologically manipulated to tolerate heat stress better by thermal conditioning/manipulation (TM) (Arjona et al., 1990; Yahav and Hurwitz, 1996). The perinatal (pre- and postnatal) period is of particular importance in the thermoregulation of poultry, because most of the functional systems, such as temperature regulation, develop during this period, from an open loop system into a closed control system. During this procedure, environmental factors may have a strong effect on the determination (imprinting) of the set-point of the thermal control system (Nichelmann, 2004). Thermosensitive neurons are located in the preoptic region of the hypothalamus. The preoptic area contains neurons that are sensitive to changes in core temperature. Preoptic thermosensitive neurons also receive somatosensory input from thermoreceptors of the skin. In this way, preoptic neurons

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compare and integrate central and peripheral thermal information (Boulant, 2000) and elicit adequate thermoregulatory responses (physiological, endocrinological, and behavioural) to keep core body temperature relatively constant. Tona et al. (2008) concluded that temperature treatment during incubation or during post-hatch life induces completely different effects on body weight and heat tolerance under heat challenge later in the life. After the first study concerning epigenetic temperature adaptation several experiments were published, but they concentrated on the performance (weight gain, egg production, feed conversion ratio etc.) of epigenetic temperature-adapted birds. However, there is a lack of research regarding the effect of heat stress on the immune responses of thermally manipulated adult birds. Tzschentke and Halle (2009) reported that short-term thermal manipulation of broiler embryos during the last four days of incubation results robust broilers, because in that period the thermoregulatory system is more responsive to 'training'. They also demonstrated that TM (+1 °C, 2 h/day from day 18 of incubation until hatching) increased the hatching rate and the weight of hatched chicks, and it resulted in better weight gain up to slaughtering age and a better feed conversion. Elmehdawi et al. (2016) reported better feed conversion in broilers as a result of the same thermal manipulation.

High environmental temperature affects the development of immune responses in chickens. These effects include a reduction in total white blood cell count (Mashaly et al., 2004), a decrease in antibody production (Beard and Mitchell, 1987; Zulkifli et al., 2000; Mashaly et al., 2004; Olfati et al., 2018) and an increase in the heterophil/lymphocyte (H/L) ratio (Maxwell, 1993; Maxwell and Robertson, 1998; Altan et al., 2000; Zulkifli et al., 2000), which is an indicator of stress (Gross and Siegel, 1983). Chicken leukocyte changes in response to stress have been found to be more reliable indicators than plasma corticosterone values (Gross and Siegel, 1983; McFarlane and Curtis, 1988). Gross and Siegel (1983) suggested that the magnitude of changes caused in the counts of heterophils and lymphocytes by feeding corticosterone is lower than that of the H/L ratio in chickens. H/L is a more sensitive indicator of stress in this species than either the heterophil or the lymphocyte change alone.

Heat stress induces immunosuppression and increases the birds' susceptibility to infectious diseases, leading to failures in the response to vaccination and immune organ involutions (Honda et al., 2015).

On the other hand, Santin et al. (2003) found that hot ambient temperature did not cause a significant alteration in antibody production against Newcastle disease virus (NDV) and infectious bursal disease virus (IBDV).

There is a scarcity of research on how the alteration of thermal conditions (in the late phase of incubation) influences the immune responses of broiler chickens subjected to heat stress at different ages.

The purpose of this study was to determine the effect of acute heat stress on the immune response (antibody production against NDV and the change in the proportions of leukocyte components) in chicks subjected to prenatal heat conditioning.

MATERIALS AND METHODS

Hatching

Eighty Ross 308 broiler chicks from the same parent stock (Bro-Ker-Bét, Hungary) were used. Two treatments were applied in the experiment: (1) control, 40 chicks hatched according to Pas Reform Hatchery Technology (36.7 °C from days 18–20 of embryonic life), and (2) 40 TM chicks subjected to thermal manipulation at 38.4 °C from days 18–20 of embryonic life; 4 h/day. The eggs were incubated in two automatic incubators (Pas Reform, The Netherlands).

Housing

The chicks were individually marked with wing bands. From hatching to day 35, the chicks were kept on deep litter according to the broiler management program of Aviagen. Feed (starter, grower and finisher) and water were provided *ad libitum*. Chickens were vaccinated by eye drop at 1 and 17 days of age with a live Newcastle disease vaccine (Avinew Neo, Merial).

Heat stress

The room temperature was decreased as recommended by the ROSS Broiler Manual. The chickens were exposed to heat challenge twice: on day 19 (31 °C, 83% relative humidity, RH/8 h, the heat index was 42 °C) and on day 35 (32 °C, 82% RH/10 h, the heat index was 42 °C).

Body weight

The birds were weighed individually every week.

Body temperature

The cloacal and back skin temperatures of 10 chickens per treatment were measured before heat stress, and in the last hour of the heat challenge. To measure the skin temperature we used an infrared thermometer (Thermoval® baby), while the cloacal temperature was measured with a VT-801SLEW thermometer.

Serum antibody titres to Newcastle disease virus (NDV) vaccine

The first immunisation was done on day 1 while the second on day 17, when NDV vaccination was repeated.

Blood samples from eight birds per experimental group were collected from the brachial vein at day 17, before the second NDV vaccination, and these samples served as the basic titres. Blood samples from eight birds per treatment were taken at day 25, after the first heat stress, and 10 samples were taken from each treatment group also at day 35, after the second heat stress.

The haemagglutination inhibition method was used for NDV antibody quantification (SRBC: Sheep Red Blood Cell, OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 2012).

Heterophil granulocytes and lymphocytes

Before the heat stress and in the last hour of heat stress blood samples were taken from the brachial vein of five birds per treatment, with EDTA being used as anticoagulant for determining the heterophil and lymphocyte counts.

Ethical issues

All protocols were approved by the Animal Protection Committee of the University of Veterinary Medicine Budapest.

Statistical analysis

The data were analysed by analysis of variance (ANOVA). The results are expressed as means ± standard error (SE). Differences were considered significant if $P < 0.05$.

RESULTS

Body weight

At the end of the experiment, both experimental groups exceeded the standard body weight. There was no difference between the treatments; the mean body weight of control chickens was 2,509 ± 257 g and that of the TM chickens was 2,498 ± 283 g.

Body temperature

During the first heat exposure (31 °C, 83% RH/8 h, the heat index was 42 °C; at day 19) the cloacal temperature did not increase either in the control or in the TM chickens. However, this heat stress significantly increased ($P < 0.05$) the back skin temperature of the TM chickens (Table 1). At day 35, the acute heat stress significantly increased both the cloacal and the skin temperature in the control as well as in the TM chickens. We observed significant differences ($P < 0.05$) in back skin temperature between the control and the TM chickens (Table 1).

Heterophil granulocytes and lymphocytes

The effect of acute heat stress on the proportion of different leukocyte subtypes is presented in Table 2. The chickens exposed to heat stress on day 19 had lower lymphocyte counts and significantly higher heterophil counts ($P < 0.05$). There was no significant difference between the treatments. The second heat stress (on day 35) did not alter the heterophil and lymphocyte profiles of chickens.

Table 1. The effects of acute heat exposure on the body temperature of 19- and 35-day-old control chickens and chickens subjected to prenatal thermal manipulation

	Control		Thermally manipulated	
	Before heat stress	After heat stress	Before heat stress	After heat stress
N	10	10	10	10
Body temperature (°C) at day 19				
Cloacal	41.39 ± 0.23	41.39 ± 0.24	41.31 ± 0.28	41.40 ± 0.28
Skin	39.35 ± 1.2^{a}	40.32 ± 0.78^{a}	39.49 ± 1.04^{a}	41.48 ± 1.00^{b}
Body temperature (°C) at day 35				
Cloacal	41.42 ± 0.20^{a}	41.85 ± 0.55^{b}	40.93 ± 0.55^{a}	41.63 ± 0.52^{b}
Skin	38.39 ± 0.81 ^{bc}	38.72 ± 0.39^{c}	37.82 ± 0.52^{a}	38.13 ± 0.72^{b}

Different superscripts (abc) within the same row indicate significant differences ($P < 0.05$).

Table 2. The effects of acute heat exposure on the proportion of leukocytes in 19- and 35-day-old control chickens and chickens subjected to prenatal thermal manipulation

	Control		Thermally manipulated	
	Before heat stress	After heat stress	Before heat stress	After heat stress
White blood cells at day 19				
Heterophils (H) (%)	34.25 ± 12.7^{a}	55.00 ± 6.6^{b}	37.08 ± 11.0^{a}	60.06 ± 7.3^{b}
Lymphocytes (L) (%)	45.52 ± 16.7	37.50 ± 4.4	48.25 ± 6.5	23.54 ± 8.2
H/L ratio	0.88	1.50	0.55	2.97
White blood cells at day 35				
Heterophils (H) (%)	43.80 ± 9.7	43.00 ± 4.9	38.40 ± 3.3	37.20 ± 5.5
Lymphocytes (L) (%)	50.40 ± 8.9	52.2 ± 3.6	54.8 ± 1.5	54.4 ± 6.2
H/L ratio	0.91	0.82	0.70	0.67

Different superscripts (ab) within the same row indicate significant differences ($P < 0.05$).

Newcastle disease virus antibody

After the first vaccination (at day 1) all of the vaccinated chickens (control and TM) presented similar positive antibody titres to ND virus on day 17 (Fig. 1).

Eight days later, after the first heat stress (31 °C, 83% RH/8 h on day 19) a considerable proportion of the samples was negative (50% in the control and 40% in the TM chickens) in spite of the second NDV vaccination performed on day 17 (Fig. 2).

After the second heat stress (32 °C, 82% RH/10 h, at day 35) the antibody titres to NDV were very low, especially in the TM chickens, with 80% of the samples being negative (Fig. 3).

DISCUSSION

The exposure of chickens to high temperature increased cloacal and skin temperatures at day 35. As expected, the rise in body surface temperature was significantly lower in the thermally manipulated broilers than in the control birds. This result is in agreement with the findings of Yahav et al. (2004) and Piestun et al. (2013). Although thermal conditioning from day 16 to day 18 of incubation improved body weight at slaughtering age but did not induce thermotolerance in chickens during a heat challenge at day 42, because the body temperatures were higher in the chicks previously conditioned at the embryonic stage (Tona et al., 2008).

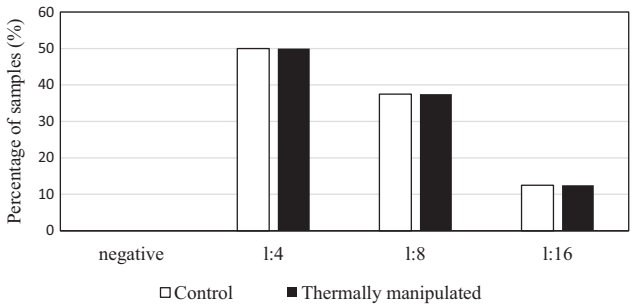

Fig. 1. Distribution of basal Newcastle disease virus antibody titres in 17-day-old chickens before heat stress (n = 8 chickens/treatment). Titres are expressed as reciprocals of the highest dilution of serum inhibiting haemagglutination

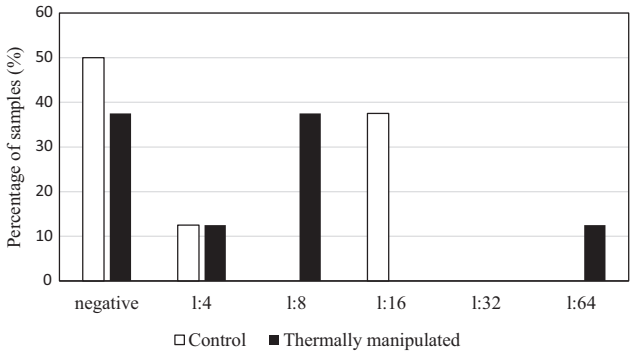

Fig. 2. Effect of heat stress (31 °C, 83% relative humidity/8 h; day 19) on the distribution of Newcastle disease virus antibody titres in 25-day-old chickens (n = 8 chickens/treatment). Titres are expressed as reciprocals of the highest dilution of serum inhibiting haemagglutination

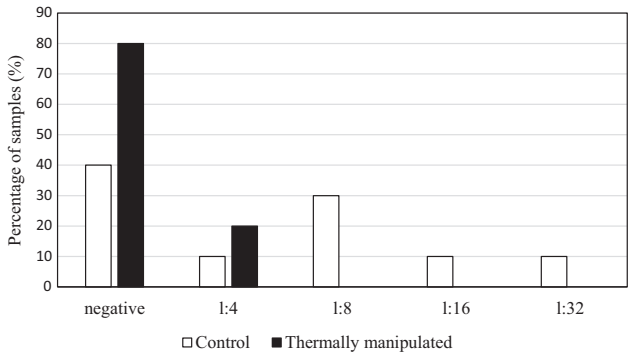

Fig. 3. Effect of heat stress (32 °C, 82% relative humidity/10 h; day 35) on the distribution of Newcastle disease virus antibody titres in 35-day-old chickens (n = 10 chickens/treatment). Titres are expressed as reciprocals of the highest dilution of serum inhibiting haemagglutination

The results of Yahav et al. (2004) show that thermal manipulation in embryonic age did not change the plasma corticosterone concentration at hatch. However, the influence of thermal challenge (at day 4) resulted in a significantly lower body temperature and plasma corticosterone concentration in the TM chicks (39.5 °C, 65% RH; 3 h/day) during the late embryogenesis (from day 16–18 of incubation) than in the control chicks.

As an important immune parameter, the ratio of circulating heterophils/lymphocytes is one of the most easily recognisable signs of stress in poultry (Gross and Siegel, 1983; Olfati et al., 2018). Wang et al. (2015) found that immunisation with different doses of an NDV vaccine did not significantly affect the heterophil/lymphocyte ratio or the proliferation of peripheral blood lymphocytes.

During the first heat challenge (31 °C, 83% RH/8 h, heat index 42 °C, at day 19) the heat stress caused changes in the circulating leukocyte components. Heat-stressed broilers responded with a reduced lymphocyte and a significantly raised heterophil count in both treatments. Therefore, the H/L ratio increased in both treatment groups.

In spite of acute heat stress during the second heat challenge (32 °C, 82% RH/10 h, at day 35), when the heat index was 42 °C, we could not find any differences induced by thermal stress in the circulating leukocyte components either in the control or in the prenatally TM chickens. Maxwell (1993) and subsequently Maxwell and Robertson (1998) suggested that an increase in the L/H ratio may be a response to mild or moderate stress but an extreme stress, such as one typical of life-threatening situations, could result in heteropenia as well as basophilia.

Our findings show that heat stress has fast and severe effects on antibody levels to Newcastle disease virus, since as

a result of the first heat stress (31 °C, 83% RH/8 h on day 19) a considerable proportion of serum samples was negative in both treatment groups (50% in the control and 40% in the TM chickens). The second heat stress (32 °C, 82% RH/10 h, on day 35) had a more serious effect on the antibody titres to NDV, particularly in TM chickens, as 80% of the samples were seronegative. Chickens in the TM group could be more susceptible to infection by NDV because they presented a reduced immune response to the vaccine.

The reduction in antibody synthesis could be indirectly due to an increase in inflammatory cytokines under heat stress. These cytokines stimulate the production of corticotropin-releasing factor and enhance corticosterone production. Corticosterone inhibits antibody production. Heat stress also decreases T-helper 2 cytokines, which are important for antibody production (Mashaly et al., 2004; Honda et al., 2015).

Honda et al. (2015) found that heat stress might guide the immune profile of chickens towards a higher percentage of T-lymphocytes, simultaneously reducing the percentage of B-lymphocytes in the peripheral blood. Their data showed that chickens vaccinated against ND presented the highest percentage of B-lymphocytes, which was expected, because NDV-vaccinated chickens predominantly develop a humoral immunity. They showed that vaccinated and heat-stressed chickens presented a decreased percentage of B-lymphocytes as compared to non-stressed vaccinated chickens. Honda et al. (2015) mention that these results point toward a stress-induced reduction in humoral activity against NDV, i.e. a decrease in immunological memory development.

The effect of corticosterone on the immune system of birds depends on the level and duration of its increase and on the components of the immune system. During the early response, as we found after the first heat challenge, corticosterone causes trafficking of lymphocytes from the blood to lymphoid tissues. At the same time, heterophils are mobilised from the bone marrow and accumulate in the blood, resulting in an elevated heterophil to lymphocyte ratio (Koutsos and Klasing, 2014). During the early response to corticosterone, both heterophils and lymphocytes increase the expression of mRNA for pro-inflammatory cytokines, resulting in a higher state of activation. Following chronic exposure to corticosterone, heterophils and lymphocytes decrease the expression of pro-inflammatory cytokines, resulting in an anti-inflammatory, immunosuppressed state during chronic stress (Koutsos and Klasing, 2014). In the present work, presumably this process may have caused the dramatic decrease of antibody titre to NDV after the first as well as the second heat exposure.

The incubation environment is very important for the development of the immune system. In the experiment of Santin et al. (2003), changes in incubation temperature (+2 °C from incubation day 13 to hatching) did not influence the humoral immune responses (the antibody titres to IBDV and NDV). At days 16, 17 and 18 of incubation and at hatch, Tona et al. (2008) found that the corticosterone levels in embryos or chicks from the TM (39.5 °C, 65% RH; 3 h/day) group were lower than in those from the control group. Combining heat conditioning at incubation and at day 3 changed corticosterone levels and body temperatures. It should be noted that the changes in corticosterone levels during pre- and post-hatch thermal conditioning showed opposite directions than in the control group. While an increase in corticosterone levels resulting from postnatal conditioning improved heat tolerance at day 42, a decrease in corticosterone levels resulting from prenatal conditioning caused a strong negative effect.

Flores et al. (2016) observed severe bursal lesions in thermally stimulated embryos (+1–1.4 °C for 2–3 h/day on days 16–18 of embryonic development). These bursal lesions presented similar characteristics as those caused by infectious bursal disease. However, the authors did not find an influence on the serum corticosterone level of chicks when embryos were subjected to a hot stimulus (+1.39 °C) as compared to the corticosterone level of chicks that had been incubated at standard incubation temperature.

From the results of our study, it can be hypothesised that thermal conditioning during incubation was not applied during a sensitive phase or by an adequate method for thermal tolerance improvement. We could not observe an improvement in deep body temperature in the TM chickens during heat challenges. The data presented in this paper show that prenatal heat stress (38.4 °C from days 18–20 of incubation, 4 h/day) impacts the development and activity of the immune system for a longer period of life, thus impairing responses to vaccination and consequently the response to pathogenic environmental challenge.

REFERENCES

Altan, Ö., Altan, A., Cabuk, M. and Bayraktar, H. (2000): Effects of heat stress on some blood parameters in broilers. Turk. J. Vet. Anim. Sci. **24,** 145–148.

Arjona, A. A., Denbow, D. M. and Weaver, W. D. Jr. (1990): Neonatally-induced thermotolerance: Physiological responses. Comp. Biochem. Physiol. **95,** 393–399.

Beard, C. W. and Mitchell, B. W. (1987): Influence of environmental temperatures on the serologic responses of broiler chickens to inactivated and viable Newcastle disease vaccines. Avian Dis. **31,** 321–326.

Boulant, J. (2000): Role of the preoptic-anterior hypothalamus in thermoregulation and fever. Clin. Infect. Dis. **31** (Suppl. 5), S157–S161.

Elmehdawi, A., Hall, M. A., Skewes, D. L., Wicker, D. L. and Maurice, D. V. (2016): Influence of thermal stimulation during the late phase of incubation on hatching results and posthatch broiler performance under commercial conditions. Br. Poult. Sci. **57,** 848–854.

Flores, F., Nääs, I. A., Garcia, R. G., Quinteiro, W. M. Q. and Souza, L. I. (2016): Effect of embryo thermal stimulation on the serum levels of immunoglobulins and corticosterone and organ histopathology of 1 day-old broilers. Br. J. Poult. Sci. Spec. Issue 2 Incubation, 71–78.

Gross, W. B. and Siegel, H. S. (1983): Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Dis. **27,** 972–979.

Honda, B. T. B., Calefi, A. S., Costola-de Souza, C., Quinteiro-Filho, W. M., da Silva Fonseca, J. G., de Paula, V. F. and Palermo-Neto, J. (2015): Effects of heat stress on peripheral T and B lymphocyte profiles and IgG and IgM serum levels in broiler chickens vaccinated for Newcastle disease virus. Poult. Sci. **94,** 2375–2381.

Koutsos, E. A., and Klasing, K. C. (2014): Factors modulating the avian immune system. In: Schat, A. E., Kaspars, B. and Kaiser, P. (eds) Avian Immunology. Second edition. Elsevier Ltd. pp. 299–313.

Mashaly, M. M., Hendricks, G. L., Kalama, M. A., Gehad, A. E., Abbas, A. O. and Patterson, P. H. (2004): Effect of heat stress on production parameters and immune responses of commercial laying hens. Poult. Sci. **83,** 889–894.

Maxwell, M. H. (1993): Avian blood leucocyte responses to stress. World's Poult. Sci. J. **49,** 34–43.

Maxwell, M. H. and Robertson, G. W. (1998): The avian heterophil leucocyte: A review. World's Poult. Sci. J. **54,** 155–178.

McFarlane, J. M. and Curtis, S. E. (1988): Multiple concurrent stressors in chicks. 3. Effects on plasma corticosterone and the heterophil:lymphocyte ratio. Poult. Sci. **68,** 522–527.

McKee, S. R. and Sams, A. R. (1997): The effect of seasonal heat stress on rigor development and the incidence of pale, exudative Turkey meat. Poult. Sci. **76**, 1616–1620.

Nichelmann, M. (2004): Perinatal epigenetic temperature adaptation in avian species: comparison of Turkey and Muscovy duck. J. Therm. Biol. **29**, 613–619.

OIE (2012): OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 2012. Chapter 2.3.14.B.2.1.2.

Olfati, A., Mojtahedi, A., Sadeghi, T., Akbari, M. and Martínez-Pastor, F. (2018): Comparison of growth performance and immune responses of broiler chicks reared under heat stress, cold stress and thermoneutral condition. Sp. J. Agricult. Res. **16**, 2.

Piestun, Y., Druyan, S., Brake, J. and Yahav, S. (2013): Thermal manipulations during broiler incubation alter performance of broilers to 70 days of age. Poult. Sci. **92**, 1155–1163.

Santin, E., Maiorka, A., Polveiro, W. J. C., Paulillo, A. C., Laurentiz, A. C., Borges, S. A. and Fischer da Silva, A. V. (2003): Effect of environmental temperature on immune response of broilers. J. Appl. Poult. Res. **12**, 247–250.

Teeter, R. G., Smith, M. O., Owens, F. N., Arp, S. C., Sanghaih, S. and Breazile, J. E. (1985): Chronic heat stress and respiratory alkalosis: Occurrence and treatment in broiler chicks. Poult. Sci. **64**, 1060–1064.

Tona, K., Onagbesan, O., Bruggeman, V., Collin, A., Berri, C., Duclos, M. C.,Tesseraud, S., Buyse, J., Decuypere, E. and Yahav, S. (2008): Effects of heat conditioning at d 16 to 18 of incubation or during early broiler rearing on embryo physiology, post-hatch growth performance and heat tolerance. Arch. Geflügelk. **72**, S75–S83.

Tzschentke, B. and Halle, I. (2009): Influence of temperature stimulation during the last 4 days of incubation on secondary sex ratio and later performance in male and female broiler chicks. Br. Poult. Sci. **50**, 634–640.

Wang, X., Zhou, Q., Shen, J., Yao, J. and Yang, X. (2015): Effect of different doses of Newcastle disease vaccine immunization on growth performance, plasma variables and immune response of broilers. J. Anim. Sci. Biotechn. **6**, 20.

Yahav, S., Collin, A., Shinder, D. and Picard, M. (2004): Thermal manipulations during broiler chick embryogenesis: Effects of timing and temperature. Poult. Sci. **83**, 1959–1963.

Yahav, S. and Hurwitz, S. (1996): Introduction of thermotolerance in male broiler chickens by temperature conditioning at an early age. Poult. Sci. **75**, 402–406.

Zulkifli, I., Norma, M. T., Israf, D. A. and Omar, A. R. (2000): The effect of early age feed restriction on subsequent response to high environmental temperatures in female broiler chickens. Poult. Sci. **79**, 1401–1407.