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# PHYSIOLOGICAL VARIATIONS DURING A GRADUAL SIX-HOUR SIMULATED HEAT STRESS IN EARLY-AGE ACCLIMATED BROILERS FED LINSEED SUPPLEMENTED DIET

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## Abstract

Panting is the first and the most visible behavioral mechanism of broilers' heat stress through which they reduce heat load by evaporation. The aim of the study was to assess the welfare of broilers reared in high ambient temperatures. It was conducted to explore the effects of broilers' thermal conditioning (TC), 5% linseed dietary supplementation (LS) and their interaction on body weight, mortality rate and some physiological variables during a simulated gradual heat wave stress hours. 400 one-day-old chicks (ISA Hubbard 15) were allotted to 4 treatments for 5 replicates of 20 chickens each as follows: The control non-treated (C), non-acclimated and fed linseed supplemented (CL), acclimated and fed standard diet (AC) and acclimated and fed linseed (ACL). To evaluate the long lasting effects of the treatments on B°C, it was recorded over the last 10 days. Birds, at local marketing age, were exposed to a simulated heat stress during which ambient temperatures were gradually (2°C/hour) increased from 30°C and maintained at 38°C for 6 hours. At each hour, respiratory rate, blood pH and rectal body temperature of 15 birds from each group were measured and recorded and, also (for the surviving birds), their correlation with final growth weight and survival rate at finish. Results show that an impaired body weight was noticed in non-treated broilers compared to the treated birds. Linseed supplementation augmented ($P<0.01$) the body weight 9.11% and TC 6.52% where their combination increased it 11.35%. TC and LS reduced ($P<0.01$) mortality in treated birds by 33.33%. Physiological improvements during the simulated heat stress hours in LS fed animals were noticed, such as decrease ($P<0.001$) in rectal body temperature at 38°C as well as the respiratory rate. Consequently, pH was improved ($P<0.001$) during all heat stress hours in treated animals compared to C. However, the association of both factors (linseed supplementation and early-age thermal conditioning) improves thermotolerance by increasing the safety fluctuation of B°C (42.20°C to 42.80°C) at 38°C compared to Control birds (45.20°C to 45.80°C). In conclusion, LS (with its bioactive-antioxidant compounds which decrease the oxidative stress) and TC improve both body weight, survival rate and animal's heat stress resistance of broilers by reducing B°C and blood pH during heat wave stress and, therefore, adverse heat stress impacts on broilers can be partially alleviated dietarily.

*Key words***:** broilers, heat stress, linseed, mortality, performance.

*List of abbreviations*:
B°C: Body temperature (°C);
RF: Respiratory frequency;
TC: Early-Age Thermal Conditioning
LS: Linseed supplementation
PUFA: Poly-Unsaturated Fatty Acid

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## Introduction

Heat stress became a serious nutritional and economic problem to broiler producers in temperate and hot areas, especially where aviculture is the mean affordable source of proteins in developing countries. However, hot climate is probably the most obvious constraint to the future development of the poultry meat industry in general but in hot countries in particular. High ambient temperature, especially when coupled with high humidity, imposes severe stress on birds and leads to increased mortality and reduced performance. Its impact on birds depends on ambient temperature, levels of relative humidity and duration of the exposure (Widowski, 2010). However, mortality due to hyperthermia occurs whether in summer environmental daily temperatures (Yahav, 2009) or during either an extended or even after a single day of a heat wave (Vale et al., 2010). Quinteiro-Filho et al. (2010) affirmed that the stress-induced hypothalamic-pituitary-adrenal axis activation by high temperatures, that is associated with an increase in corticosterone (Gomes et al., 2014), is responsible for the negative effects observed on the chickens' performance, immune function and the changes of the intestinal mucosa.

Poultry and particularly commercial broilers are known to be highly sensitive to temperature–associated environmental challenges (Bhadauria et al., 2014). According to Suganya et al. (2015) birds perform better in a relatively extended range of ambient temperature (from 10°C to 27°C), where the optimal temperature for the finishing period is from 20°C to 25°C (Yahav, 1998). Although effective cooling structures and equipment could be installed in poultry houses to make birds more comfortable under heat stress, they are not always economically suitable due to their high cost (Ojano-Dirain and Waldroup, 2002; Tirawattanawanich et al., 2010). However, less expensive cooling installations may be affordable such as fans, evaporative pads and cooling perches which ameliorate heat loss through sensible means (conduction and convection).

Panting is the first and the most important visible behavioral mechanism of broilers in high ambient temperatures through which the animal reduces the heat load by evaporation. However, heat-stressed broiler has to make physiological adaptations to meet and dissipate the extra heat load it is generating. Nevertheless, evaporation increases respiratory frequency which, in turn, causes higher $CO_2$ losses and, obviously, results in altered homeostatic acid-base balance and increased blood pH which may leads to alkalosis. This change is proportional to change in core body temperature (especially in heavier and older birds). Thus, when ambient temperature exceeds the thermoneutral level, it may lead to higher body temperature and would increase the production of lactate in muscle, which in turn increases the rate of pH decline and subsequently affects broilers' meat quality (Zhang et al., 2012).

Extensive trials, in the last two decades, have investigated the heat stress-adverse constraints and their impact on broilers' performance, physiological responses and meat quality (Petracci et al., 2001; Lu et al., 2007; Soleimani et al., 2011; Quinteiro-Filho et al., 2010; Quinteiro-Filho et al., 2012; Bengharbi et al., 2014; Lowman et al., 2014; Chen et al., 2015). Moreover, numerous attempts have been made and many nutritional strategies have been experienced to alleviate heat stress and ameliorate chicken thermoresistance and, hence, improve the global animal's production (Hurwitz et al., 1987; Picard et al., 1993; Tesseraud and Temim, 1999; Collier et al., 2006; Lin et al., 2006; Zulkifli et al., 2007; Ahmad et al., 2008; Ghazalah et al., 2008; Daghir, 2009; Hyun-Seok et al., 2012; Zhang et al., 2012, Chen et al., 2015; Khosravinia et al., 2015).

Early age thermal conditioning of broiler chicks seemed to be a reliable manipulation which stimulates broilers' thermoresistance, either on farm (De Basilio et al., 2001a) or at experimental station (De Basilio et al., 2001b) levels. It enhances the adaptability of broilers to heat stress conditions (Yahav and Hurwitz 1996; Arjona et al., 1988; Zhou et al., 1997; Yahav and Plavnik, 1999).

Increasing the energy content of the diet can partially overcome growth depression, especially by adding fat. However, high fat diets (5%) help in reducing the detrimental effect of heat stress in broilers raised in high temperatures (29-36° C) due to the reduced heat production, since fat has a lower heat increment than either proteins or carbohydrates (Ghazalah et al., 2008). Recent studies have focused on the use of phytogenic feed additives to alleviate negative effects of heat stress in broiler chickens (Suderman and Solikhah, 2011 ; Zeinali et al. 2011; Khosravinia et al., 2013; Quiroga and Asensio, 2015).

The linseed in broilers diet is an important source of essential oils rich in phenols and lignans compounds which improve antioxidant defense against detrimental adverse heat stress impacts (Akbarian et al., 2014; Huang et al., 2015). It is also a source of fat and has been preferred for its high contents of linolenic acid which is the precursor for the n-3 PUFA synthesis. The latter, however, can affect the carcass composition by reduction of body fat (Newman et al., 2002; Ferrini et al., 2008; Wongsuthavas et al., 2008).

This study was conducted to investigate, on one hand, the effects of early age thermal conditioning and 5% ground linseed (*Linum usitatissimum*) dietary supplementation and their combination on some physiological variables: body temperature (B°C); respiratory frequency (RF) and blood pH during the gradual simulated heat stress hours. On the other hand, it aims to highlight the chronological variations of these variables. Moreover, in order to estimate their correlations with the animals' thermal resistance, body weights and mortality were registered at the end of the trial.

## Material and Methods

***Animals and experimental design***

400 one-day-old chicks (ISA Hubbard 15) were obtained from a local commercial hatchery.

They were weighed and equally distributed to 20 experimental units in a randomized blocks design and provided with *ad-libitum* access to water and commercial starter meat-type chicken diet. At 5 days of age, 50% of the pens were thermally conditioned (TC: exposed to 39±1°C for 24 hours) and also, at the end of the starter phase, 50% of the experimental birds were fed 5% supplemented dietary ground linseed (LS). However, all birds were allotted to 4 treatments for 5 replicates of 20 chickens each: The control non-treated (C), non-acclimated and fed 5% linseed supplemented diet (CL), acclimated and fed standard diet (AC) and acclimated and fed linseed supplemented diet (ACL). All experimental chickens were reared (according to the European legislation for the protection of animals used for scientific purposes) in an ambient, unless stated, temperature of 21±1°C.

***Acquisition***

Over ten days, prior to the heat stress, daily body temperatures of 15 birds from each treatment were recorded from d-43 to d-52 of age. Pens, at local marketing age (53 days), were exposed to a heat stress of 37±1°C for 6 hours. However, during the simulated heat stress hours, ambient temperatures were gradually (2°C/hour) increased: 30°C, 32°C, 34°C, 36°C, and finally maintained at 38°C for 6 hours. At each hour, respiratory rate (per minute), blood pH and core body temperature (Lowe et al., 2007; Quimby et al., 2009; Lohse et al., 2010) of 15 birds from each treatment, randomly selected, were measured and recorded. Except mortality and body weights which were registered at the end of the trial, all variables were recorded while reaching the final heat temperature.

***Feeding diets***

Standard meat-type diets and linseed (*Linum usitatissimum*) were purchased from a local livestock food industry. Composition and nutritive metabolizable energy of the experimental diets (Table 1) were assessed and balanced. Feedstuff and levels of metabolizable energy of starter, grower and linseed were calculated (Carpenter and Clegg, 2006).

**Table 1**
**Composition and calculated analyses of the experimental diets (S: starter, G: grower and 5% LS: 5% linseed supplemented diet)**

| Feedstuff composition | S | G | 5%LS |
|---|---|---|---|
| Ingredient, % | | | |
| Yellow Corn | 22 | 24 | 23 |
| Wheat | 39.8 | 40.6 | 36.56 |
| Soyabean meal | 29.1 | 27.50 | 27.50 |
| Wheat Bran | 5.15 | 4.74 | 4.74 |
| Linseed | 0 | 0 | 5 |
| Methionine | 0.03 | 0.00 | 0.04 |
| MVC[1] | 1 | 1 | 1 |
| Anti stress | 1 | 0 | 0 |
| Calculated analyses[2] | | | |
| ME (Kcal/Kg) | 2887.06 | 2887.06 | 2887.06 |
| Moisture % | 11.59 | 10.62 | 10.62 |
| CP % | 21.86 | 19.95 | 20 |
| Ether extract % | 2.68 | 2.86 | 4.91 |
| Ash (% of DM) | 6.97 | 6.52 | 6.52 |
| Fibre (% DM) | 2.87 | 3.02 | 4.62 |
| Calcium % | 1.22 | 1.31 | 1.31 |
| Available P % | 0.69 | 0.63 | 0.63 |

[1] Supplied per kilogram of diet: *7000 IU, vit A; 1400 ICU, vit D3; 20 IU, vit E; 50 mg, vit C; 2.3 mg, vit K; 1.8 mg, vit B1; 5.5 mg, vit B2; 2.3 mg, vit B6; 0.011 mg, vit B12; 27.5, mg Niacin; 0.90 mg, Folic acid; 7 mg, PA; 0.092 mg, Biotin; 50 mg, Antioxidant; 8.5mg, copper; 0.35 mg, Iodine; 0.26 mg, Iron; 0.45 mg, Manganese; 0.2 mg, Selenium; 45 mg, Zinc.*

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***Statistical analysis***

Data were analyzed by two-way analysis of variance by the ANOVA procedure. The statistical evaluation was done by JMP v. 7 software package.

## Results and Discussion

***Body temperatures prior to the heat stress***

Over 10 days and starting from 42-d old, as shown in Table 2, rectal body temperatures (B°C) were numerically higher in CL and ACL compared to C and AC. They fluctuated between 40.85°C and 41.05°C. These preliminary data showed that the linseed supplementation increases, in an overall manner, the B°C of treated pens at different age of finish. Moreover; early age thermal conditioning (TC) increased (P<0.05 and

P<0.01) B°C on d-45 and d-46 respectively. Nevertheless, linseed supplementation increased (P<0.01) B°C on d-48 and on the last day before the simulated heat stress (d-52). However, the interaction of the thermal conditioning and the linseed diet was noticed (P<0.01) on d-44 and d-45 of age.

***B°C during the gradual simulated heat stress hours***

In non-thermally conditioned birds (C and CL), increasing the ambient temperature during the simulated heat stress was accompanied by a highly significant (P<0.001) gradual increase in B°C reaching a maximum of 45.80°C and 45.20°C at 38°C respectively (Table 3). Whereas, thermal adaptation of the thermally treated pens (AC and ACL) was observed, where maximal B°C at the most heat stressful ambient temperatures were 42.80°C and 42.20°C respectively. However, supplementation of linseed to the diet affected the B°C leading to a significant (P<0.05) decrease at 34°C compared to the control (43.90°C). The TC and LS interaction was the strongest (P<0.001) at the highest experimental ambient temperature (48°C).

***Respiratory response to heat stress***

As panting or increased respiratory rate (respiratory frequency: RF) is one of the most visible symptoms of a bird under heat stress. Obtained data (Table 4) show clearly the effectiveness (P<0.05 and P<0.001) of the early age acclimation

**Table 2**
**Impact and interaction of early age thermal conditioning and linseed dietary supplementation on rectal temperature of the experimental birds from d-43 to d-52 of age reared at 21±1°C**

| Age, days | Core body °C | | | | RMSE | Significance | | |
|---|---|---|---|---|---|---|---|---|
| | C | CL | AC | ACL | | TC | LS | TCxLS |
| 43 | 40.82 | 40.87 | 40.90 | 40.91 | 0.22 | | | |
| 44 | 41.06 | 40.94 | 40.86 | 41.01 | 0.14 | | | ** |
| 45 | 40.82 | 40.99 | 40.87 | 40.79 | 0.11 | * | | ** |
| 46 | 40.99 | 41.03 | 40.89 | 40.90 | 0.12 | ** | | |
| 47 | 40.88 | 40.91 | 40.93 | 40.85 | 0.11 | | | |
| 48 | 40.79 | 40.96 | 40.84 | 40.98 | 0.13 | | ** | |
| 49 | 40.88 | 40.96 | 40.86 | 40.90 | 0.15 | | | |
| 50 | 40.96 | 40.95 | 40.84 | 40.97 | 0.19 | | | |
| 51 | 40.89 | 41.05 | 40.89 | 40.89 | 0.18 | | | |
| 52 | 40.79 | 40.97 | 40.88 | 41.00 | 0.14 | | ** | |

TC: thermal conditioning; LS: flaxseed dietary supplementation; C: control-untreated animals;
CL: non-acclimated animals fed linseed; AC: acclimated animals; ACL: acclimated animals fed linseed; RMSE: root mean square error;
* p<0.05; ** p<0.01; *** p<0.001

**Table 3**
**Impact and interaction of early age thermal conditioning and linseed dietary supplementation on rectal temperature of the experimental birds during the gradual heat stress hours**

| Ambient °C | Body °C | | | | RMSE | Significance | | |
|---|---|---|---|---|---|---|---|---|
| | C | CL | AC | ACL | | TC | LS | TCxLS |
| 22°C | 40.79 | 40.97 | 40.88 | 41.00 | 0.15 | ** | | |
| 30°C | 42.80 | 42.70 | 41.00 | 41.20 | 0.35 | *** | | |
| 32°C | 42.90 | 42.70 | 41.60 | 41.40 | 0.30 | *** | | |
| 34°C | 43.90 | 43.60 | 42.80 | 42.62 | 0.23 | *** | * | |
| 36°C | 44.70 | 44.80 | 42.00 | 42.20 | 0.31 | *** | | |
| 38°C | 45.80 | 45.20 | 42.80 | 42.20 | 0.29 | *** | *** | |

TC: thermal conditioning; LS: flaxseed dietary supplementation; C: control-untreated animals; CL: non-acclimated animals fed linseed; AC: acclimated animals; ACL: acclimated animals fed linseed; RMSE: root mean square error;
* p<0.05; ** p<0.01; *** p<0.001

during all the tested heat stress temperatures where increased panting was pronounced and accompanied all the gradual increase of ambient temperatures stages. However, the respiratory adaptation of birds, showed by increased respiratory frequency (RF), was noticed in the acclimated birds (AC and ACL) from the first hour (123/min and 126/min vs 120/min and 119/min; P<0.001) of the heat stress compared the non-thermally acclimated pens (C and CL) respectively.

Although there were different values of the effect of the linseed dietary supplementation over the heat stress hours, it was significant (P<0.05) only at 34°C. RF was increased in birds fed LS and TC leading to a better physiological comfort where the combination of the two treatments was more visible.

***Homeostatic blood pH balance***

Compared to the 22°C, effect of the linseed supplementation was well pronounced over all the increasing heat stress ambient temperatures (Table 5). The combination of LS and TC showed a better adaptation of birds to the acid-base balance. However, ACL birds resisted better to the pH increase (7.10/22°C; 7.34/32°C and 7.48/48°C) compared to the untreated birds (7.50/22°C; 7.60/32°C and 7.80/48°C). Nevertheless, the effect of separate treatments was better in the pH increase resistance for LS followed by the TC.

***Mortality rate and Body weight***

After the six hours of the heat stress (38°C), mortality rate and body weight of the surviving birds of the four treatments were recorded. Obtained results show that high temperature can have a dramatic effect on mortality and growth of chickens. TC and LS reduced mortality in treated birds by 33.33%. However, mortality rate was the highest (P<0.01) among the control population (C) where it reached 20% compared to 5% in the other three treatments. Surviving treated birds were active and in a better welfare comfort compared to C individuals. There was a significant interaction of both factors in the response of the populations to heat stress. An impaired body weight was noticed in non-treated broilers compared to the treated birds. Linseed supplementation augmented the

**Table 4**
**Effects of gradual increasing heat stress ambient temperatures on TC and LS broilers' panting rate**

| Ambient °C | RF/minute | | | | RMSE | Significance | | |
|---|---|---|---|---|---|---|---|---|
| | C | CL | AC | ACL | | TC | LS | TCxLS |
| 22°C | 92 | 95 | 101 | 103 | 2.64 | *** | | |
| 30°C | 120 | 119 | 123 | 126 | 3.53 | *** | | |
| 32°C | 169 | 165 | 163 | 162 | 3.50 | * | | |
| 34°C | 172 | 176 | 171 | 172 | 2.52 | * | * | |
| 36°C | 198 | 199 | 186 | 186 | 3.44 | *** | | |
| 38°C | 232 | 233 | 248 | 249 | 2.64 | *** | | |

TC: thermal conditioning; LS: flaxseed dietary supplementation; C: control-untreated animals; CL: non-acclimated animals fed linseed; AC: acclimated animals; ACL: acclimated animals fed linseed; RMSE: root mean square error;
* p<0.05; ** p<0.01; *** p<0.001

**Table 5**
**Effects of early age TC and LS on respiratory rate of broilers while increasing the heat stress ambient temperatures**

| Ambient °C | Blood pH | | | | RMSE | Significance | | |
|---|---|---|---|---|---|---|---|---|
| | C | CL | AC | ACL | | TC | LS | TCxLS |
| 22°C | 7.50 | 7.16 | 7.40 | 7.10 | 0.26 | | * | |
| 32°C | 7.60 | 7.39 | 7.58 | 7.34 | 0.07 | | *** | |
| 34°C | 7.68 | 7.48 | 7.59 | 7.47 | 0.09 | | ** | |
| 36°C | 7.80 | 7.50 | 7.62 | 7.48 | 0.24 | | * | |
| 38°C | 7.89 | 7.51 | 7.67 | 7.50 | 0.14 | | *** | |

TC: thermal conditioning; LS: flaxseed dietary supplementation; C: control-untreated animals; CL: non-acclimated animals fed linseed; AC: acclimated animals; ACL: acclimated animals fed linseed; RMSE: root mean square error
* p<0.05; ** p<0.01; *** p<0.001

body weight 9.11% and TC 6.52% where their combination increased it 11.35%. Thus, data show that studied factors (TC and LS) increased (P<0.01) body weight of the treated groups (ACL, CL and AC) compared to C (2276±22g, 2230±28g and 2157±34g vs 2044±31g, respectively).

## Discussion

Broilers, like all other avian species, are homoeothermic warm-blooded birds. Thus, in nature, they have the ability to cope and maintain a relatively stable internal body temperature under extreme environmental conditions. Nevertheless, when ambient temperature exceeds the tolerable level, heat loss by non-evaporative means is reduced and, then, welfare problem exist. As a result, B°C would begin to raise leading to hyperthermia where its increase by 4°C is usually considered as life-threatening to Birds (DEFRA, 2005). Therefore, estimating B°C of the stressed birds could be a useful welfare indicator.

Nevertheless, as found by several authors (Yahav, 1998; Olanrewaju et al., 2010; Bhadauria et al., 2014; Suganya et al 2015), our results (Table 2) show that when broilers reared at the comfort ambient temperature (21±1°C), core body temperature of the four treatments birds during the last ten days of finish phase was in the normal range and fluctuated between 40.79°C and 41.06°C. In these optimum conditions where B°C is nearly two times the ambient temperature, minimal metabolic effort is needed to lose or produce heat. As a result, the thermal comfort participates to maintain the control of homeostatic processes involved in the regulation of core body temperature as suggested by Sherwood et al. (2005). It has well been established that the heavier the bird, the more difficult it becomes to dissipate heat. However, even in thermal comfort, linseed supplemented diet increased (P<0.01) B°C of birds at the 52$^{nd}$ day of age (though in the normal range) which was, probably, due to the nutritional values of the linseed and to the noticed difference in body weights as linseed birds were heavier.

During the simulated increasing of the gradual heat stress, compared to comfort temperature, B°C were increased (P<0.001) in control birds (Table 3). This relationship agrees with reported conclusions of several similar studies (Donkoh, 1989; Berrong and Washburn, 1998; Cooper and Washburn, 1998; Jingjing et al., 2015). They reach crucial values of 45.80°C and 45.20°C in C and CL compared to acclimated birds 42.80°C and 42.20°C in AC and ACL respectively. Therefore, early-age acclimation effects on B°T were very pronounced compared to the LS. Thus, thermoresistance of the acclimated birds (AC an ACL) were showed over all the increased temperatures and even at the highest (38°C) and adaptability of birds was expressed by decreased B°T. It is associated with better resistance of individuals at even hotter climate late in life. These results are similar to those obtained in different studies either through manipulation during embryogenesis (De Basilio et al., 2001a; De Basilio et al., 2001b; Tona et al., 2008; Piestun et al., 2013) or thermal conditioning at early ages (Arjona et al., 1988; Yahav and Plavnik, 1999; Yahav and McMurtry, 2001; Tona et al., 2008). However, the combination of both factors (LS and TC) showed an even better influence on B°C in ACL birds which was 42.20°C vs 42.80°C at 38°C compared AC pens. Increasing B°T leads to higher free radical production and subsequently oxidative damage occurs.

Panting (increased respiratory rate) is the first and the most important visible behavioral mechanism of broilers in high ambient temperatures through which the animal reduces the heat load by evaporation (evaporative cooling). The gradual increase of ambient temperatures during the simulated gradual heat stress was accompanied by an increase in respiratory frequency (Table 4). TC effects were also well pronounced during all the experienced temperatures compared to the second factor (LS). Nevertheless, there were decreased respiratory rates of C and CL (232/min and 233/min) compared to AC and ACL (248/min and 249/min) at 38°C respectively. Associated with increased B°T in C and CL and decreased in AC and ACL means that only TC and its association with linseed diet would alleviate heat stress effects. Early-age TC improves the cardio-respiratory system and the linseed diet with its bioactive compounds. Thus, in response to heat stress birds increase respiratory rate which, in turn, increases the exchange in the lungs for CO2 leading to a decrease in pCO2 and consequently, to an increase in bicarbonate (HCO3-) which will be excreted by the kidneys. The combination of both treatments has positive effect on increasing RF and at the same time decreasing the B°T of the acclimated and fed linseed individuals (AC and ACL). It was noticed that RF data, even, at the comfort ambient temperatures (21±1°C) all treated birds followed nearly the same increasingly ranking in RF at different ambient temperatures of the heat stress (C followed by CL, AC and finally ACL). However, there was no significant interaction between the two-studied factors similar to what was shown in their effects on B°T.

Blood pH control is insured by the lungs, kidneys and the various buffer systems, which prevent rapid changes in the pH, but its increase may leads to alkalosis. In this study, blood pH was improved (P<0.001) during all heat stress hours in the linseed dietary fed birds. In control birds, when ambient temperature settled at 38°C, blood pH was 7.89 (i.e., alkalosis) which caused high mortality (20%) compared to (25%) found by Abdelqader and Al-Fataftah (2014). Linseed supplementation showed significant positive effects Ground linseed supplemented diet with its bioactive molecules such as omega 3 and omega 6 unsaturated fatty acids, lignans and essential oils helped in reducing the detrimental effect of heat stress in broilers and, consequently, contribute to dissipate metabolic heat. This resistance may be due to the rich linseed antioxidant compounds which may decrease the oxidative stress. It confers to birds the ability to alter their behavior seeking thermoregulation by decreasing body temperature.

It is well-documented that high environmental conditions have negative and sometimes devastating effects on poultry production and, in particular, broiler intensive rearing. Evaporation needs increased respiratory frequency which, in turn, causes higher $CO_2$ losses and, obviously, results in altered homeostatic acid-base balance and increased blood pH (i.e., alkalosis). Results indicated that, dietary linseed, as natural feed, inclusion to broilers reared in high ambient temperature or exposed to a heat shock wave at the most economically and critical stage of life 'finisher phase' is of positive effects. This treatment caused a significant increase ($P<0.01$) in body weigh compared to the untreated group, and a considerable decrease ($P<0.01$) in mortality rate. Series of physiological improvements during the simulated heat shock hours were recorded such as decreased ($P<0.05$ and $P<0.001$) body temperature at 34°C and 38°C respectively. It slightly reduced the respiratory rate at 34°C where blood pH was improved ($P<0.001$) during all heat stress hours.

Association of both factors (linseed supplementation and early-age thermal conditioning) improves thermotolerance by increasing the safety fluctuation of B°T (42.20°C to 42.80°C) at 38°C compared to Control birds (45.20°C to 45.80°C). Ground linseed supplemented diet with its bioactive molecules such as omega 3 and omega 6 unsaturated fatty acids, lignans and essential oils helped in reducing the detrimental effect of heat stress in broilers and, consequently, contribute to dissipate metabolic heat. This resistance may be due to the rich linseed antioxidant compounds which may decrease the oxidative stress.

## Conclusion

In conclusion, the 5% linseed dietary inclusion offers a reliable nutritional strategy for broilers reared in hot climate. Its combination with the early age thermal conditioning technique enhances more broilers' thermal resistance adaptability. It can be applied by industrial farmers in hot climate countries to alleviate the tremendous economic losses caused by heat stress and to maintain growth performance, meat quality and improve thermal resistance and hence the animal's welfare.

However, this area of linseed diet inclusion to early thermal conditioned birds still requires further investigation to highlight the molecular effects of the linseed protective bioactive compounds on detrimental adverse heat stress impacts and to characterize the basic physiological mechanisms and increase the actual knowledge.

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## References

**Abdelqader, A. and A. R. Al-Fataftah,** 2014. Thermal acclimation of broiler birds by intermittent heat exposure. *Journal of Thermal Biology*, **39**(1): 1-5.

**Ahmad, T., T. Khalid, T. Mushtaq, M. A. Mirza, A. Nadeem, M. E. Babar and G. Ahmad,** 2008. Effect of potassium chloride supplementation in drinking water on broiler performance under heat stress conditions. *Poultry Science,* **87:** 1276–1280.

**Akbarian, A., J. Michiels, A. Golian, J. Buyse, Y. Wang and S. De Smet**, 2014. Gene expression of heat shock protein 70 and antioxidant enzymes, oxidative status, and meat oxidative stability of cyclically heat-challenged finishing broilers fed Origanum compactum and Curcuma xanthorrhiza essential oils. *Poult Sci.* 93(8): 1930-41. doi: 10.3382/ps.2014-03896.

**Arjona, A., D. Denbow and W. Weaver.** 1988. Effect of heat stress early in life on mortality of broilers exposed to high environmental temperatures just prior to marketing. *Poult.Sci.,* **67**: 226–231.

**Bhadauria, P., J. M. Kataria, S. Majumdar, S. K. Bhanja and K. G. Divya,** 2014. Impact of Hot Climate on Poultry Production System-A Review*, Journal of Poultry Science and Technology,* **2**: 56-63.

**Bengharbi, Z., S. Dahmouni, A. Mouats and M. Halbouche,** 2014. Effet d'un traitement thermique précoce d'une semaine à température décroissante sur l'évolution du poids vif du poulet de chair élevé en climat chaud. *European Scientific Journal,* **10** (12): 30-45. (Fr)

**Berrong, S. L. and K. W. Washburn,** 1998. Effects of genetic variation on total plasma protein, body weight gains, and body temperature responses to heat stress. *Poultry Science*, **77**: 379–385

**Carpenter, K. J. and K. M. Clegg**, 2006. The metabolizable energy of poultry feeding staffs in relation to their chemical composition, *J. Sci. Food and Agriculture*, **7**(1): 45–51.

**Chen, J., G. Tellez, J. D. Richards and J. Escobar,** 2015. Identification of potential biomarkers for gut barrier failure in broiler chickens. *Front. Vet. Sci.* **2**: 14.

**Collier, R. J., G. E. Dahl and M. J. VanBaale,** 2006. Major advances associated with environmental effects on dairy cattle. *J. Dairy Sci.,* **89**: 1244–1253.

**Cooper, M. A. and K. W. Washburn,** 1998. The Relationships of Body Temperature to Weight Gain, Feed Consumption, and Feed Utilization in Broilers under Heat Stress. *Poultry Science,* **77**: 237–242

**Daghir, N. J.,** 2009. Nutritional strategies to reduce heat stress in broilers and broiler breeders. *Lohmann Information,* **44**, 6–15.

**De Basilio, V., I. Oliveros, M. Vilarino, J. Diaz, A. Leon, and M. Picard**. 2001a. Intérêt de l'acclimatation précoce dans les conditions de production des poulets de chair au Venezuela. *Rev. Elev. Med. Vet. Pays Trop.***, 54**: 159–167.

**De Basilio, V., M. Vilarin, A. Leon and M. Picard**. 2001b. Efecto de la aclimatacion precoz sobre la termotolerancia en pollos de engorde sometidos a un estrestermico tardıo en condiciones de clima tropical. *Rev. Cientıfica, FCV-LUZ.*, **11**: 60–68.

**DEFRA**, 2005. Heat stress in Poultry- solving the problem. *Department for Environment, Food and Rural Affairs,* 1-20.

**Donkoh, A.,** 1989. Ambient temperature: a factor affecting performance and physiological response of broiler chickens. *Int. J. Biometeorol*., **33**: 259–265.

**Ferrini, G., M. D. Baucells, E. Esteve-Garcia and A. C. Barroeta,** 2008. Dietary polyunsaturated fat reduces skin fat as well as abdominal fat in broiler chickens. *Poultry Science,* **87**: 528–535

**Ghazalah, A. A., M. O. Abd-Elsamee and A. M. Ali,** 2008. Influence of dietary energy and poultry fat on the response of broiler chicks to heat stress. *International Journal of Poultry Science,* **7**(4)**:** 355-359.

**Gomes, A. V., W. M. Quinteiro-Filho, A. Ribeiro, V. Ferraz-de-Paula, M. L. Pinheiro, E. Baskeville, A. T. Akamine, C. S. Astolfi-Ferreira, A. J. Ferreira and J. Palermo-Neto,** 2014. Overcrowding stress decreases macrophage activity and increases Salmonella Enteritidis invasion in broiler chickens. *Avian Pathol*.*,* **43**(1): 82-90.

**Huang, C., H. Jiao, Z. Song, J. Zhao, X. Wang and H. Lin,** 2015. Heat stress impairs mitochondria functions and induces oxidative injury in broiler chickens. *J. Anim. Sci*.**, 93**(5): 2144-53. doi: 10.2527/jas.2014-8739.

**Hurwitz, S., I. Plavnik, I. Rosenberg, I. BenGal, H. Talpaz and I. Bartov**, 1987. Differential response to dietary carbohydrates and fat of turkeys kept at various environmental temperatures. *Poultry Science*, **66**: 1346–1357.

**Hyun-Seok Chae, Hee Chul Choi, Jae Cheon Na, Min Ji Kim, Hwan Ku Kang, Dong Wook Kim, Ji Hyeok Kim, Soo Hyun Jo, Chong Eon Lee, Nam Young Kim, Yang Ho Choi and Byong Sung Park**, 2012. Effect of electrolytic material feeding on blood and carcass traits of broiler under intense heat condition in summer. *Korean J. Poult. Sci.*, **39** (3): 183–193.

**Jingjing Xie, Li Tang, Lin Lu, Liyang Zhang, Xi Lin, Hsiao-Ching Liu, Jack Odle and Xugang Luo**, 2015. Effects of acute and chronic heat stress on plasma metabolites, hormones and oxidant status in restrictedly fed broiler breeders. *Poultry Science*, **94** (7): 1635-1644, http://dx.doi.org/10.3382/ps/pev105

**JMP,** 2007. Version 7, SAS Institute Inc. Cary, NC.

**Khosravinia, H., M. Alirezaei, S. Ghasemi and S. Neamati,** 2015. Effect of *Satureja khuzistanica* essential oils on antioxidative potential and postmortem pH of breast muscle in heat stressed broiler chicken. *Journal of Veterinary Research,* Vol. **70** (2): 227–234.

**Lin, H., H. C. Jiao, J. Buyse and E. Decuypere,** 2006. Strategies for preventing heat stress in poultry. *World's Poult. Sci. J.,* **62**: 71–86.

**Lohse, L., A. Uttenthal, C. Enoe and J. Nielsen,** 2010. A study on the applicability of implantable microchip transponders for body temperature measurements in pigs. *Acta Veterinaria Scandinavica,* **52:** 29.

**Lowe, J. C., S. M. Abeyesinghe, T. G. M. Demmers, C. M. Wathes and D. E. F. McKeegan,** 2007. A novel telemetric logging system for recording physiological signals in unrestrained animals. *Computers and Electronics in Agriculture*, **57** (1): 74-79.

**Lowman, Z. S., F. W. Edens, C. M. Ashwell and S. J. Nolin,** 2014. Actigen Influence on the Gene Expression of Heat® Shock Proteins in Ross 708 Broiler Chickens. *International Journal of Poultry Science* **13** (2): 114-123,

**Lu, Q., J. Wen, and H. Zhang,** 2007. Effect of chronic heat exposure on fat deposition and meat quality in two genetic types of chicken. *Poult. Sci.,* **86**: 1059–1064

**Newman, R. E., W. L. Bryden, E. Fleck, J. R. Ashes, W. A. Buttemer, L. H. Storlien and J. A. Downing,** 2002. Dietary n-3 and n-6 fatty acids alter avian metabolism: Metabolism and abdominal deposition. *Br. J. Nutr.,* **88**(1)**:** 11-8.

**Ojano-Dirain, C. P. and P. W. Waldroup, 2002.** Protein and amino acid needs of broilers in warm weather: A Review'. *International Journal of Poultry Science*, **1**: 40-46.

**Olanrewaju, H. A., J. L. Purswell, S. D. Collier and S. L. Branton,** 2010. Effect of ambient temperature and light intensity on growth performance and carcass characteristics of heavy broiler chickens at 56 days of age. *International Journal of Poultry Science,* **9**(8): 720-725.

**Petracci, M., D. Fletcher, and J. Northcutt,** 2001. The effect of holding temperature on live shrink, processing yield, and breast meat quality of broiler chickens. *Poult. Sci.,* **80**: 670–675.

**Picard, M., B. Sauveur, F. Fernadji, I. Angulo and P. Mongin,** 1993. Ajustements technico-économiques possibles de l'alimentation des volailles dans les pays chauds. *INRA Production Animale,* **6**: 87–103.

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

**Quimby, J. M., F. Olea-Popelka and M. R. Lappin,** 2009. Comparison of digital rectal and microchip transponder thermometry in cats. *Journal of the American Association for Laboratory Animal Science,* **48**(4): 402-404.

**Quiroga, P. R. and C. M. Asensio,** 2015. Antioxidant effects of the monoterpenes carvacrol, thymol and sabinene hydrate on chemical and sensory stability of roasted sunflower seeds. *J Sci Food Agric.,* **95**(3): 471–479

**Quinteiro-Filho, W. M., A. Ribero, V. Ferraz-de-Paula, M.L, Pinheiro, M. Sakai, L.R.M. Sa, A.I.P. Ferreira and J. Palermo-Neto,** 2010. Heat Stress impairs performance parameters, induces intestinal injury and decreases macrophage activity in broiler chickens. *Poult. Sci.,* **85**: 1905-1914.

**Quinteiro-Filho, W. M., M. V. Rodrigues, A. Ribeiro, V. Ferraz-de-paula, M. L. Pinheiro, L. R. M. Sa, A. J. P. Ferreira, and J. Palermo-Neto,** 2012. Acute heat stress impairs performance parameters and induces mild intestinal enteritis in broiler chickens: Role of acute HPA axis activation. *J. Anim. Sci.,* **90**: 1986–1994.

**Sandercock, D. A., R. R. Hunter, G. R. Nute, M. A. Mitchell and P. M. Hocking,** 2001. Acute heat stress-induced alterations in blood acid-base status and skeletal muscle membrane integrity in broiler chickens at two ages: Implications for meat quality. *Poultry Science,* **80**(4): 418-425.

**Sandercock, D. A., R. R. Hunter, M. A. Mitchell and P. M. Hocking,** 2006. Thermoregulatory capacity and muscle membrane integrity are compromised in broilers compared with layers at the same age of body weight. *British Poultry Science,* **47**(3): 322-329.

**Sherwood, L., H. Klandorf and P. H. Yancey,** 2005. Animal Physiology: From Genes to Organisms, 840.

**Soleimani, A. F., I. Zulkifli, A. R. Omar and A. R. Raha,** 2011. Physiological response of three breeds to acute heat stress. *Poult. Sci.,* **90**: 1435-1440.

**Suderman, A. and S. H. Solikhah,** 2011. Performance and meat cholesterol content of broiler chickens fed Pluchea indica L. leaf meal reared under stress condition. *Media Peternakan, hlm*. pp. 64–68

**Suganya T., S. Senthilkumar, K. Deepa and R. Amuth,** 2015. Nutritional management to alleviate heat stress in broilers. *International Journal of Science, Environment and Technology,* **4**(3): 661 – 666

**Tesseraud, S. and S. Temim,** 1999. Modifications métaboliques chez le poulet de chair en climat chaud: conséquences nutritionnelles. *INRA Production Animales*, **12:** 353–363.

**Tirawattanawanich, C., S. Chanatakru, W. Nimitsantiwong and S. Tongyai,** 2010. The effects of tropical environmental conditions on the stress and immune responses of commercial broilers, Thai Indigenous chickens, and crossbred chicken. *Journal of Applied Poultry Research*, **20**(4): 409-420.

**Tona, K., O. Onagbesan, V. Bruggeman, A. Collin, C. Berri, M. J. Duclos, S. Tesseraud, J. Buyse, E. Decuypere and S. Yahav,** 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** (2): 75–83.

**Vale, M. M., D. J. Moura, I. A. Naas and D. F. Pereira,** 2010. Characterization of heat waves affecting mortality rates of broilers between 29 days and market age. *Revista Brasileira de Ciencia Avicola,* **12**(4): 279-285.

**Widowski, T.,** 2010. The physical environment and its effect on welfare. In: Duncan, I. J. H. and Hawkins, P. (ed.) *The welfare of Domestic Fowl and other Captive Birds*. *New York*: Springer, pp. 149-159

**Wongsuthavas, S., S. Terapuntuwat, W. Wongsrikeaw, S. Katawatin, C. Yuangklang and A. C. Beynen,** 2008. Influence of amount and type of fat on deposition, adipocyte count and iodine number of abdominal fat in broiler chickens. *Poultry Science,* **87**: 528–535

**Yahav, S.,** 1998. The effects of acute chronic heat stress on performance and physiological responses of domestic fowl. *Trends comp. Bochem. Physiol.,* **5**: 187-199.

**Yahav, S.,** 2009. Alleviating heat stress in domestic fowl: Different strategies. *World's Poultry Science Journal,* **65**: 719-732.

**Yahav, S. and S. Hurwitz,** 1996. Induction of thermotolerance in male broiler chickens by temperature conditioning at an early age. *Poultry Science,* **75**: 402-406.

**Yahav, S. and J. P. McMurtry,** 2001. Thermotolerance acquisition in broiler chickens by temperature conditioning early in life—the effect of timing and ambient temperature. *Poult. Sci.* **80**: 1662–1666.

**Yahav, S. and I. Plavnik.** 1999. Effect of early-age thermal conditioning and food restriction of performance and thermotolerance of male broiler chickens. *Br. Poult. Sci.,* **40**: 120–126.

**Zeinali, A., H. Kermanshahi, A. Riasi, H. Farhangfar, H. Sarir and H. Ziaie,** 2011. Effects of sodium selenite and turmeric powder on thyroid hormones and plasma lipids of broiler chickens reared under heat stress condition. *Glob Vet.,* **6:** 237–240

**Zhang, Z. Y., G. Q. Jia, J. J. Zuo, Y. Zhang, J. Lei, L. Ren and D. Y. Feng,** 2012. Effects of constant and cyclic heat stress on muscle metabolism and meat quality of broiler breast fillet and thigh meat. *Poultry Science,* **91**: 2931–2937

**Zhou, W., M. Fujita, T. Ito and S. Yamamoto,** 1997. Effects of early heat exposure on thermoregulatory responses and blood viscosity of broilers prior to marketing. *Br. Poult. Sci*., **38:** 301–306.

**Zulkifli, I., N. Htin, A. R. Alimon, T. C. Loh and M. Hair-Bejo,** 2007. Dietary selection of fat by heat-stressed broiler chickens. *Asian-Australasian Journal of Animal Science*, **20:** 245–251.