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**ORIGINAL ARTICLE**

# Impact of Cold Stress on Physiological, Hormonal and Immune Status in Male and Female Broad Breasted White Turkeys

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In this study physiological, certain blood hormones and immune status of male and female Broad Breasted White turkeys were recorded under cold stress. Birds were acclimated two weeks prior to the start of experiment and later divided into two groups. Control group (n=12 with equal number from each sex) was maintained at an environmental temperature of 27-30 °C and Test group (n=12 with equal number from each sex) was housed in a designed chamber were the temperature (10±1°C) remained stable at least for 5-6 hours in a day. After 3 consecutive days (72 hours) of temperature treatment Phytohaemagglutinin-P (PHA-P) at the dose rate of 0.5 mg in 0.1 ml PBS were injected to the patagium of both control and test group of birds to study the cell mediated immune response by measuring the dermal swelling in response to inflammatory reaction after 24 hours of injection of Phytohaemagglutinin-P (PHA-P). After 96 hours of cold treatment the blood samples were collected from the wing vein to analyze the blood hormonal levels using standard protocols. The physiological parameters like respiration rate, rectal temperature and surface temperature were recorded at the morning hours of the day during experimental period. Significant difference ($p \leq 0.01$) between treatments were observed in body temperatures, respiration rate, Heterophil%, H/L ratio, Wing web thickness, T4 and cortisol levels.

*Key words: Broad breasted white turkeys, cold stress, hormones, immunity*

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Physiological tolerance of organisms is strong determinant of the environmental conditions in which they inhabit. At certain range of environmental temperature the organisms maintain a normal body temperature with least involvement of thermoregulatory mechanism. This range of ambient temperature is called a zone of thermo neutrality. The environmental temperature beyond the upper and lower limit of thermo-neutral zone is supposed to produce heat or cold stress in animals. Physiological stress that originates from adverse climatic condition may have profound economic

influence in organisms effecting reproductive or productive efficiency including health and disease resistant capacity. Adverse climatic conditions may occur as random events or as unusual changes. Exposure of poultry birds to extreme temperature stressor modulates the immune responsiveness and haemato-biochemical parameters of birds (Hangalapura *et. al.,* 2004). Among all the environmental stressors, cold stress induces physiological responses which are of high priority and energy demanding in homeotherms.

## MATERIALS AND METHODS

The experiment was carried out at the Division of Veterinary Physiology, F.V.Sc and A.H, SKUAST-J R.S Pura. To induce cold stress in birds an experimental chamber (not exactly a psychrometric chamber) was created in Division of Veterinary Physiology. The temperature of microclimate of the room was maintained at 10±1°C with the use of air conditioner &/or room cooler.

Twenty four (24) Broad Breasted White turkeys of 20 weeks of age from both sexes in equal numbers were selected randomly from a large group of birds basing on their weight, size and behavior for the experiment. The experiment was repeated twice during consecutive weeks using 6 birds of each sex in each group (n=12). Cold stressed birds were housed in the designed chamber were the required temperature (10±1°C) remained stable for at least 5-6 hours in a day during the cold stress treatment. After 72 hours of temperature treatment to assess cell mediated immunity an intradermal injection of Phytohaemagglutinin-P ( PHA: a plant lectin which causes dermal infiltration of lymphocytes and phagocytes) at the rate of 0.5 mg of PHA-P dissolved in 0.1 ml of PBS was given. After four consecutive days (96 hours) of temperature treatment birds were weighed and hormonal parameters, immune status assessed by in vivo and in vitro assays. Blood samples were collected from the wing vein of birds using 10ml disposable syringe. The blood was centrifuged at 3000rpm for 30 minutes to collect plasma in sterile eppendorff tubes for biochemical analysis. T3, T4, TSH and cortisol level of blood of control as well as cold stressed birds were analysed using the 'immuno chemiluminiscence microparticle assay (ICMA).

The respiration rate was measured by keeping hand on thoraco abdominal region with slight pressure and respiration rate was recorded by observing thoraco abdominal movements per minute and expressed in number of respiration per minute (King and Molony, 1971). Body Surface and Rectal temperatures were recorded during the morning hours of the day during experimental period. Body surface temperature were recorded using the clinical thermometer at two regions of body i.e from surface of head (featherless) and below the wings and expressed in °F.

The Rectal temperature was recorded by inserting a clinical thermometer into the rectum approximately 2-3 inches deep. The bulb of the thermometer is kept for at least 2-3 minutes inside the rectum ensuring that the bulb of the thermometer was in contact with the rectal wall. The recording of the temperature was recorded in °F.

**Statistical Analysis:**

The data generated during the experiment were used to calculate mean standard error and multi trait ANOVA by using the Systat software (2007) 32-bit Unicode English version 17.02.0.

## RESULTS AND DISCUSSION

Rectal Temperature is considered as a good indicator of core body temperature because of its close proximity to the abdominal cavity and longer equilibrium period. The mean rectal temperature as per (Table.1) in males of control group was 103.58°F and that in females under the control group was 103.43°F. The rectal temperature of males and females after cold stress were 104.72°F and 104.97 respectively. It is clear from (table. 1) that sex has no significant effect on rectal temperature whereas the treatments (cold stress) significantly ($p \leq 0.01$) affected the rectal temperature of birds. After 1st cold stress it has been observed that the rectal temperature increased up to 104.72°F in male and 104.97°F in females. This slight increase in body temperature might be due to increase in the metabolic activity in response to non shivering thermogenesis and to maintain the thermal homeostasis as evident from increase in the metabolic hormone status during the cold stress.

Surface temperature of the body is closely related with the temperature of the micro-environment as well as the body covering. The head and wing temperature of the males and females (Table.1) under control group were 100.23°F, 101.58°F and 100.23°F, 101.50°F respectively. The recorded values after cold stress were 98.18°F, 100.48°F and 98.08 °F, 100.88°F respectively in male and female birds. The surface temperature of the turkeys (head and below wing) was reduced in comparison to the normal as a result of decrease in temperature of the microclimate as well as less blood flow to the skin in order to check the heat dissipiation from body to the environment. This finding is in agreement with the work of Marchand and Walker, 1996. The head and wing temperature did not differ significantly between sex (Table 1) but the treatments influenced the parameter significantly ($p \leq 0.01$).

The respiration rate of birds is normally influenced by environmental temperature. Table 1 reveals drastic reduction in the respiration rate from 43.67/minute and 41.17/minute in males and females under control group to 38/minute and 38.08/minute in both the sexes under cold stress group respectively. The respiration rate has been observed to decline from control to cold stress groups in order to check the convective heat loss from body to the environment , as 1 gram of vapour in expired air is responsible for loss of 0.536 K Cal of heat from body to the environment. The reduced blood flow to different body parts and suppressed tissue metabolism during initial part of cold stress (Marchand and Walker, 1996) might be another reason for the decrease in respiration rate.

T3 levels in males were 0.56 and 0.60 ng/ml and in females were 0.56 and 0.63 ng/ml under control and cold stress groups respectively (Table 2). T3 levels very significantly ($p \leq 0.01$) under control and cold stress groups. T4 levels in males were 1.13 and 1.78 ng/ml and in females were 1.13 and 1.73 ng/ml under control and cold stress groups respectively (Table 2). TSH levels in males were 0.03 and 0.05 ng/ml and in females were 0.04 and 0.04 ng/ml under control and cold stress groups respectively (Table 2). T4 and TSH levels very significantly ($p \leq 0.01$) under control and cold stress groups. Triiodo thyronine (T3) influence food intake and weight gain and its concentration in the blood affected by the ambient temperature. Stojevic *et al.* 2000 reported that T3 concentration in plasma is inversely related to the environmental temperature. The concentration of hormone T3 observed during the present study in control and cold stress groups were found to be lower than the

values observed by Yahav, 2002. The findings of the present study that increase in T4 and T3 levels after acute cold stress is supported by the findings of Blahova (2007). But the present finding is contradicted by the previous report of Hangalapura 2004 that chronic cold stress suppresses the thyroid activity and shortest duration of cold stress significantly increase the T3 level.

Cortisol concentration under control and cold stress groups were 0.26, 2.62 µg/dl and 0.25, 2.18 µg/dl in males and females respectively (Table 2). Analysis of variance shows that sex has no effect on cortisol levels, whereas treatment affected cortisol levels at highly significant level ($p \leq 0.01$). The cortisol level could be used as an indicator of stress as evident from the present study. The increase in corticosterone in response to acute cold stress might be caused by partial habituation coping and adaptive but the variation was very wide between sexes which is supported by Hester *et al* 1996. But Hanglapura *et al* 2004a reported a suppresive effect of cold stress on plasma corticosterone level which is in disagreement with present finding.

**Table 1.** Body temperatures and respiration rates of turkeys during normal and cold stress periods

| Parameters | Control | | Cold Stress | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| Head Temp. ($^0$F) | 100.23± 0.38$^a$ | 100.23 ± 0.33$^a$ | 98.18 ± 0.35$^b$ | 98.08 ± 0.38$^b$ |
| Wing Temp. ($^0$F) | 101.58± 0.40$^a$ | 101.50 ± 0.30$^a$ | 100.48 ± 0.51$^b$ | 100.88 ± 0.16$^b$ |
| Rect. Temp. ($^0$F) | 103.58± 0.42$^a$ | 103.43 ± 0.61$^a$ | 104.72 ± 0.35$^b$ | 104.97 ± 0.45$^b$ |
| Resp. Rate (No/minute) | 43.67± 0.76$^a$ | 41.17 ± 0.75$^a$ | 38.00 ± 0.53$^b$ | 38.08± 0.35$^b$ |

Mean under different superscript in the same row differ significantly at $p \leq 0.01$

**Table 2.** Hormonal values of turkeys during normal and cold stress periods

| Parameters | Control | | Cold Stress | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| T3 | 0.56 ± 0.06$^a$ | 0.56 ± 0.02$^a$ | 0.60 ± 0.02$^b$ | 0.63 ± 0.04$^b$ |
| T4 | 1.13 ± 0.06$^a$ | 1.13 ± 0.04$^a$ | 1.78 ± 0.19$^b$ | 1.73 ± 0.12$^b$ |
| TSH | 0.03 ± 0.01$^a$ | 0.04 ± 0.01$^a$ | 0.05 ± 0.01$^b$ | 0.04 ± 0.01$^b$ |
| Cortisol | 0.26 ± 0.01$^a$ | 0.25 ± 0.01$^a$ | 2.62 ± 0.09$^b$ | 2.18 ± 0.15$^b$ |

Mean under different superscript in the same row differ significantly at $p \leq 0.01$

**Table 3.** Immunological values of Turkeys during normal and cold stress periods

| | Control | | Cold stress | |
|---|---|---|---|---|
| Parameters | Male | Female | Male | Female |
| H/L | 0.6± 0.03$^a$ | 0.57 ± 0.02$^a$ | 0.34 ± 0.04$^b$ | 0.39 ± 0.03$^b$ |
| Wing web thickness | 3.5 ± 0.06$^a$ | 3.17 ± 0.21$^a$ | 4.92 ± 0.16$^b$ | 4.34± 0.16$^b$ |

Mean under different superscript in the same row differ significantly at $p \leq 0.01$

Heterophil/Lymphocyte (H/L) ratio is a good indicator of stress in poultry birds. From the study it was observed that H/L ratio decreased after cold stress than that in the control group. The same trend was observed in both male and female birds which are shown in tabular form in Table 3. From the analysis of variance it is observed that the temperature treatment has highly significant effect ($p \leq 0.01$) on H/L ratio. H/L ratio increased to peak after short term cold stress (6°C for 30 minutes) and came back to normal after 1 day from cold stress (Gross WB, 1989). In present observation it was found that H/L ratio declined from control to cold stress groups which implies that cold stress has immune modulatoy effect in birds. The result of present study was well supported by Hanglapura *et. al.,* (2004) that cold stress stimulates both innate and parts of adaptive cellular immune system. The proliferation of lymphocyte number during cold stress is also supported by Sinclaire *et. al.,* (2000) suggesting the enhancement in immune responsiveness.

The inflammation followed by intradermal injection of PHA-P acts as a good indicator of cell mediated immunity. The wing web thickness in response to PHA-P injection increased in cold stressed groups. The observed values in both male and female have been reflected in Table 3. The wing web thickness after intradermal injection of PHA-P is highly significantly ($p \leq 0.01$) influenced by both sex and temperature treatments. The inflammation following the injection of phytohaemagglutinin-p into the patagium or wing web of birds of cold stress group was found to be more prominent than control when compared with the wing web thickness of PBS injected site. This indicates an enhancement in cell mediated immunity characterized as localized reaction of lymphocytes and phagocytes to pathogens as the PHA causes dermal infiltration by lymphocytes and phagocytes.

## CONCLUSION

It may be concluded from the present study that there is a definite effect of cold stress on physiological, hormonal status and immune responsiveness in male and female broad breasted white turkeys. Cold stress has modulating effect on cellular immunity as indicated by decreased H/L ratio and increased wing web thickness. Other biochemical parameters do also change in response to cold stress helping birds to overcome stressful conditions of cold. Moreover further study is needed regarding the adaptation to cold stress in relation to thermoregulation and immunity.

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