

## **EFFECT OF FEED WITHDRAWAL AND DARKENING PERIODS ON PHYSIOLOGICAL AND IMMUNOLOGICAL RESPONSES OF MUSCOVY DUCKS DURING HIGH ENVIRONMENTAL TEMPERATURE**

**By**

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**ABSTRACT:** *A total of 180 one day-old Muscovy ducklings were used to assess the effect of feed withdrawal and darkness on, triiodothyronine (T<sub>3</sub>) hormones, some plasma metabolites, immune response, respiration rate and body temperature in Muscovy ducks during summer season. The ducklings were distributed randomly into three equal experimental groups, each one had 60 ducks. The first group was used as control. Ducklings in the second group (feed withdrawal) were subjected to feed removal from 11:00 to 16:00h, and ducklings in the third group (darkening) were subjected to light restriction from 11:00 to 16:00h*

*Feed withdrawal and darkening treatments improved the respiration rate, body temperature and immune responses than the control. Plasma levels of triiodothyronine (T<sub>3</sub>) were not affected by feed removal and darkness period. There was no significant differences in plasma total protein, globulin and albumin concentration among groups, but plasma creatinin, and uric acid values in feed withdrawal and darkening groups were decreased significantly as compared to control group,*

### **INTRODUCTION**

In birds the optimum ambient temperature during the growing period ranges from 20-25 °C, but elevation of temperature above 30 °C from 4 weeks of age up to the market age will reduce rate of growth, feed intake and feed conversion ratio (Meltzer, 1987). Kamar (1962) stated that Pekin, Rouen and Khaki Campbell ducks had light body weight in Egypt due to the unfavorable environmental conditions including high air temperature. It was proved that a temperature of 23-25 °C has been demonstrated to result in maximum growth of 1-7 weeks old ducks and an increase in environmental

the temperature above 29 °C decreased the performance of ducks (Wilson *et al.*, 1980 and Hester *et al.*, 1981).

High ambient temperature decreases the rate of heat dissipation, which led to elevate body temperature (Yahav and Hurwitz, 1996). Accordingly, birds minimize their endogenous heat production by reducing feed intake to reduce body temperature. resulting in decreasing growth and meat yield in broilers (Yahav and Hurwitz, 1996).

Feed withdrawal during the hottest hours of the day has become a common practice in many broiler producing areas. Short-term feed withdrawal can lower the bird's body temperature and increase its ability to survive during acute heat stress (Smith and Teeter, 1988). Heat production and rectal temperature were reduced by controlled feeding. However, McCormick *et al.*, (1980) demonstrated that feed remove for 24, 48, 72h produced lessens the heat load and enhance the survival during heat stress.

Melatonin in birds is secreted mainly during darkness (Pang *et al.*, 1996). Melatonin plays an important role in circadian thermoregulatory adjustments of body temperature. Melatonin reduces heat production by lowering body temperature and regulating heat dissipation (Shoukry *et al.*, 1993; Rozenboim *et al.*, 1998; Apeldorn *et al.*, 1999 and Zeman *et al.*, 2001). Also, Melatonin may act by lowering the central set point temperature, thereby causing immediate stimulation of heat loss mechanisms.

The objective of the present study was to compare the duration of feed withdrawal and darkening during high environmental temperature condition and their effect on thermal reaction, some physiological and immunological responses in duck.

## **MATERIAL AND METHODS**

The experiment was carried out in farm private farm in the province Kalyobiya. The experiment was carried out during summer months (from 7th of July to 14th of September 2007).

### **Birds, diet and treatments:**

One hundred and eighty one-day old Muscovy ducklings were used in the present study to explain the effect of feed withdrawal and darkening on ducklings under heat stress conditions during summer season (The ambient temperature ranged between 26.5 - 38 °C and relative humidity 55-65%),

All ducklings were wing banded and individually weighed. The experiment consisted of three groups. Each group included three replicates of 20 birds each. The birds were housed in floor pens. Each replicate was kept in a partition of 3 square meter provided with litter of wheat straw (3 cm depth). The birds of all groups were maintained under continuous lighting with water available all the time and the windows were covered by black sheets. The first group was used as control (C) which was fed *ad libitum* throughout the experimental period (from one day to 10 weeks of age). Ducklings in the second group (feed withdrawal) were fed *ad libitum* but it were subjected to feed removal for 5 hours per day (from 11.00 a.m. to 16.00 p.m then return it after that all day) from 4 to 10 weeks of age and ducklings in the third group (darkening) were subjected to light restriction 5 hours per day (from 11.00 a.m. to 16.00 p.m then return it after that all day whereas, lights were switched off during this period) from 4 to 10 weeks of age. During the first 14 days, all birds were fed on a starter diet containing 2900 Kcal ME/kg and 20% crude protein. At the beginning of the third week of age, all birds were fed on a growing diet containing 2700 Kcal ME/kg and 15.5% crude protein. All diets met the nutrient requirements of Muscovy ducklings and were formulated according to NRC (1994).

### **Measurements**

#### **Rectal Temperature (RT) and Respiration Rate (RR):**

Nine birds were randomly taken from each group (3 birds / replicate / group) for measuring rectal temperature and respiration rate. These parameters were measured just before treatment period termination at 10 weeks of age. Rectal temperature was obtained gently by thermometer inserted into the cloaca and respiration rate by counting the body wall movement for one minute using stop watch and a counter.

#### **Plasma parameters:**

Blood samples (about 3ml heparinized blood / bird) at the end of the experiment were collected at the afternoon during the period from 1 to 3 o'clock from 9 birds per treatment taken randomly from the brachial vein just before treatment period termination at 10 weeks of age. Blood samples were divided into two portions. The first blood portion was immediately centrifuged at 3000 rpm for 15 minutes and plasma was then separated and stored at -20°C for later analysis and the second portion was used to measure the Hemoglobin (Hb) (g/dl) and packed cell volume (PCV) (%).

Plasma samples were analyzed for total protein concentrations according to Henry *et al.* (1974); Plasma albumen was determined

according to Doumas (1971) and plasma globulin was obtained by subtracting the values of the albumen from the corresponding values of the total protein Aspartate-aminotransferase (AST), Alanine-aminotransferase (AL T) activities were determined using the method described by Reitman and Frankel (1957) Creatinine and uric acid were determined using the method described by Husdan, (1968) and Bogin and Keller (1987), respectively.

#### **Hormonal assay:**

Plasma concentrations of triiodothyronine ( $T_3$ ) hormone were determined by radioimmunoassay technique with commercial kits (Medical Technology, USA). Sensitivity values for  $T_3$  measured to be 7ng / ml approximately. Hormone levels were determined according to the procedure outlined by the manufacturer.

#### **Hematological parameters**

The second blood portion of ducks was taken to determine haemoglobin (g/dl) and packed- cell volume (%). Haematocrit value was determined and expressed as a percentage of packed cell volume (PCV %) according to Hunsaker (1969). Haemoglobin concentration was determined in fresh blood samples using haemoglobinometer according to Pilaski (1972). Also, Red blood cells and White blood cells were counted in fresh blood samples using hemocytometer and light microscope at 10 weeks of age.

. Two hundred white blood cells were counted and differentiated into Lymphocytes (L) and Heterophils (H), and H/L ratio was calculated. Both the two slides were counted and the mean of H/L ratio was calculated for each birds.

#### **Immunization and Titration**

At 10 weeks of age 9 birds / group were injected into-muscular with 0.5 ml of 10% suspension packed sheep red blood cells (SRBC). Pre-injection antibody titers were zero. Blood samples were collected at three and seven days post immunizing with SRBC to determine the primary antibody response. Antibody levels quantities using micro titration hemeagglutination technique. Van der ziipp and Leenstra (1980). Antibody titer values expressed as log 2 of the highest dilution giving total agglutination.

#### **Statistical analysis:**

Data were statistically analyzed using one way analysis of variance by using SAS, Institute, Inc. (2001). The statistical model was as follows:

$$Y_{ijk} = \mu + T_i + e_{ijk}$$

**Where:**  $Y_{ijk}$  = Observation of the duck;  $\mu$  = Overall mean, common element to all observations;  $T_i$  = Effect of the feed withdrawal and darkness period ( $i = 1, 2, 3$ ); and  $e_{ijk}$  = Random error component assumed to be normally distributed. Data estimated in percentage were transformed with the arcsine square-root procedure to normalize variance before analysis and were retransformed again to the original scale before presentation. The differences among means were tested using Duncan's New Multiple Range Test (Duncan, 1955).

## RESULTS AND DISCUSSION

### Rectal Temperature (RT) and Respiration Rate (RR):

As shown in Table (1) feed removal and darkness period during hot climate, resulted in a significant reduction in RT compared to control group.

These results are in agreement with those of Francis et al., (1991) who found that feed removal for 2h before the hot period and no light (Darkness) extinguished throughout the hot period led to a significant reduction in rectal temperature. Wiermusz and Teeter (1993) reported that feed withdrawal benefits heat stress broiler by lowering heat production.

Moreover, melatonin plays an important role in circadian thermoregulatory adjustments of body temperature. Melatonin reduces heat production which led to lowering body temperature and regulating heat dissipation (Apeldorn et al., 1999 and Zeman et al., 2001). Also, melatonin may act by lowering the central set point temperature, thereby causing immediate stimulation of heat loss mechanisms.

Also, the same trend was observed for RR whereas, feed removal and darkness period during hot climate, resulted in a significant reduction in RR compared to control group. These results are in accordance with those of Rozenboim et al. (1998) who concluded that pharmacological doses of melatonin induce hypothermia in hens by increasing non-evaporative skin heat losses and slightly increasing respiratory evaporation.

Similarly, Abd El-Hakim et al., (1986) and Khalil and Abd El-Hakim (1990) concluded that feed withdrawal under heat stress caused a reduction in respiration rate because of reduction in metabolic rate due to the reduction in oxygen consumption and accordingly reduction in the movement of respiratory muscles.

These results may suggest that feed removal and darkness period released tranquilizing effects on birds by depressed their activities during hot weather resulting in lowering body temperature and better body heat dissipation.

#### **Some Blood Constituents:**

Data in Table (1) showed that plasma T3 concentration was not affected by feed removal and darkness period whereas, data revealed no significant differences in plasma T3 concentration among groups.

These results are in agreement with those of Gonzales et al., (1998) suggested that short-term fasting did not affect on T3 levels may be due to a very fast adaptive thyroid hormone response to feed restriction.

Data in Table (2) revealed no significant differences in plasma total protein, globulin and albumin concentration among groups, These results are disagreement with those obtained by Sahin and Kucuk (2001) who found that darkening and feed withdrawal treatments increased serum total protein and albumin concentrations in laying hens under heat stress.

#### **Enzymes activities:**

The effects of feed removal and darkness period during hot climate on liver function are shown in Table (3). Results showed that plasma AST and ALT values were not affected by feed removal and darkness period wheaeas, there were no significant differences in plasma AST and ALT values among groups.

These results are in agree with those results obtained by Sahin and Kucuk (2001) who found that darkening and feed withdrawal treatments did not influence the activities of serum AST and ALT in laying hens under heat stress.

The average values of liver enzyme activity were almost within the normal range indicating that all treatments had no deleterious effects on liver function.

On the other hand, the effects of feed removal and darkness period during hot climate on kidney function are shown in Table (3). Results showed that plasma creatinin and uric acid values were affected by feed removal and darkness period whereas, there was significant decrease ( $P>0.05$ ) in plasma creatinin and uric acid values in treated groups compared to control group. These results are disagreement with those findings obtained by Sahin and Kucuk (2001) who found that darkening and

feed withdrawal treatments increased serum uric acid concentrations in laying hens under heat stress.

### **2-1 Hematological parameters**

The effects of feed removal and darkness period during hot climate on hematological parameters are shown in Table (4). Results showed that red blood cells counts were not affected by feed removal and darkness period whereas, there were no significant differences in red blood cells among groups.

Also, Zhou et al., (1998) who found that red blood cells count was greater during the dark than during the light period.

Results in Table (4) showed that hemoglobin concentration was not affected by feed removal and darkness period whereas; there were no significant differences in hemoglobin concentration among groups.

These results are in consistent with that reported by De-Jong et al., (2002) who postulated that fasted birds showed spent more time for drinking. Water consumption lead to hemodilution (Sturkie, 1986) and there is a positive relation between the RBC's numbers and hemoglobin concentration in blood (Vo et al., 1978 and Sturkie, 1986)

Results in Table (4) showed that were affected by feed removal and darkness period whereas, there were significant differences in hematocrit values among groups.

Feed withdrawal and darkening groups had recorded higher ( $P < 0.05$ ) value in hematocrit than the control group. These results agree with results obtained by Sahin and Kucuk (2001) who found that hematocrit values were higher in darkening and feed withdrawal groups compared with control group under heat stress. Also, these results are in agreement with those results obtained by Zhou et al., (1998) who found that hematocrit values were greater during the dark than during the light period.

The lower hematocrit values could be attributed to the increase of water consumption of feed-restricted or darkening birds which consequently decreased both the blood viscosity and the hemoconcentration and thus reduced the hematocrit values (Hocking, et al., 1996). Whole blood viscosity increased linearly with hematocrit values (Zhou et al., 1998). Increased blood viscosity caused by changes in the properties of the plasma and / or the cellular components may effectively alter peripheral resistance and thus tissue perfusion and circulatory distribution including the supply to heat exchange surfaces (Hocking et al., 1994).

The effects of feed removal and darkness period during hot climate on hematological parameters are shown in Table (5). Results showed that white blood cells counts and lymphocytes were not affected by feed removal and darkness period whereas, there were no significant differences in white blood cells and lymphocytes among groups.

The opposite trend in heterophil and H/L ratio whereas, heterophil and H/L ratio in feed withdrawal and darkening groups had recorded lower ( $P < 0.05$ ) value than the control group. These results are agreement with those obtained by Moore and Siopes (2000) and Brennan *et al.* (2002) they found that decreasing the photoperiod or adding melatonin to the drinking water of Japanese quail significantly increased lymphocyte percentage, decreased heterophil percentage, and decreased the H/L ratio. This depression in these parameters for treatment may be due to less activity when lights were switched off which associated with secretion of melatonin from pineal gland. Injidi and Forbes (1983)

The effects of feed removal and darkness period during hot climate on antibody titer against SRBC's are shown in Table (5). That at three days post immunization feed removal and darkness period did not affect on immune response. But the primary immune responses against SRBC's increased and reach it highest level at seven days post immunization in darkening group following by feed withdrawal group whereas, Yamamoto and Glick (1982) and Ahmed (1999) found that the primary antibody against SRBC's was maximizing at the 6<sup>th</sup> and 7<sup>th</sup> day post immunization, respectively.

Most photoperiod and dark studies on immune function to date show an increase in immune response in short compared with long days. Cockerels grown under constant lighting had a lower anti-SRBC (Sheep Red Blood Cells) titer than those grown under 12 h light: 12 h darkness (Kirby and Froman, 1991). Also, Kliger *et al.* (2000) indicated that the intermittent photoperiod regimen, indirectly through melatonin, enhances immune function of broiler chickens when compared with constant lighting.

**Table (1): Effect of feed withdrawal and darkening period on respiration rate and rectal temperature, of Muscovy ducks during summer season.**

Treatment Item	Control	Feed withdrawal	Darkening	SE
Respiration Rate (R.R.)	71.66 <sup>a</sup>	65.00 <sup>b</sup>	62.33 <sup>b</sup>	2.01
Rectal temperature (R.T) <sup>°C</sup>	42.04 <sup>a</sup>	40.52 <sup>b</sup>	40.68 <sup>b</sup>	0.39

<sup>a,b,c</sup> Means with different superscripts in the same row within item differ significantly (P<0.05).

**Table (2): Effect of feed withdrawal and darkening period on some blood metabolites and T3 concentration of Muscovy ducks during summer season.**

Treatment Item	Control	Feed withdrawal	Darkening	SE
Total protein g/dl	5.06	5.75	5.84	0.29
Albumin (A) g/dl	2.81	3.22	3.17	0.17
Globulin (G) g/dl	2.26	2.53	2.67	0.32
T3 concentration ng / ml	0.66	0.76	0.78	0.04

<sup>a,b,c</sup> Means with different superscripts in the same row within item differ significantly (P<0.05).

**Table (3): Effect of feed withdrawal and darkening period on creatinin, uric acid, AST and ALT values of Muscovy ducks during summer season.**

Treatment Item	Control	Feed withdrawal	Darkening	SE
Creatinin mg/100ml	0.52 <sup>a</sup>	0.36 <sup>b</sup>	0.26 <sup>c</sup>	0.02
Uric acid mg/100ml	5.71 <sup>a</sup>	4.16 <sup>b</sup>	3.31 <sup>c</sup>	0.24
AST U/I	150.55	149.65	149.63	2.43
ALT U/I	13.08	13.02	12.97	0.41

<sup>a,b,c</sup> Means with different superscripts in the same row within item differ significantly (P<0.05).

**Table (4): Effect of feed withdrawal and darkening period on Hematological parameters of Muscovy ducks during summer season.**

Item \ Treatment	Control	Feed withdrawal	Darkening	SE
RBC's $\times 10^6$ mm <sup>3</sup>	2.51 <sup>a</sup>	2.54 <sup>a</sup>	2.67 <sup>a</sup>	0.13
HB	9.72	9.32	9.14	0.48
PCV %	25.58 <sup>b</sup>	30.25 <sup>a</sup>	28.75 <sup>a</sup>	1.14

<sup>a,b,c</sup> Means with different superscripts in the same row within item differ significantly (P<0.05).

**Table (5): Effect of feed withdrawal and darkening period on Hematological parameters of Muscovy ducks during summer season.**

Item \ Treatment	Control	Feed withdrawal	Darkening	SE
WBC's $\times 10^3$ mm <sup>3</sup>	26.75	28.92	30.34	1.24
Heterophil (H)%	31.05 <sup>a</sup>	26.78 <sup>b</sup>	24.58 <sup>b</sup>	1.14
Lymphocytes (L)%	59.42	62.53	68.54	3.67
H/L%	0.52 <sup>a</sup>	0.42 <sup>b</sup>	0.39 <sup>b</sup>	0.01
Primary antibody at 3 days	4.53	4.48	4.46	0.58
Primary antibody at 7 days	7.59 <sup>c</sup>	10.19 <sup>b</sup>	11.86 <sup>a</sup>	0.56

<sup>a,b,c</sup> Means with different superscripts in the same row within item differ significantly (P<0.05).

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## الملخص العربي

### تأثير فترات منع التغذية والاضلام على الاستجابات الفسيولوجية والمناعية في البط المسكوفي اثناء درجات حرارة البيئة العالية

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مركز البحوث الزراعية - وزارة الزراعة - الدقى - ج.م.ع.

أجريت هذه الدراسة في فترة شهور صيف 2007 وذلك لدراسة تأثير فترة منع التغذية او الاضلام اثناء درجات حرارة البيئة العالية على الاستجابات الفسيولوجية والمناعية للبط المسكوفي وكذلك معدل التنفس ودرجة حرارة الجسم.

أستخدم في هذه الدراسة 180 كتكوت من البط المسكوفي عمر يوم وقسمت إلى ثلاثة معاملات متساوية المجموعة الأولى استعملت ككونترول وتم فيها تغذية الطيور الى حد الشبع من عمر يوم وحتى عمر 10 اسابيع أما المجموعة الثانية تم سحب الغذاء لمدة ساعات كل يوم من عمر 4 حتى 10 اسابيع اما المجموعة الثالثة تم اضلام العنبر بدون سحب الغذاء وذلك لمدة ساعات كل يوم من عمر 4 حتى 10 اسابيع وكانت أهم النتائج كالآتي:-

كلا من مجموعة المنع الغذائي ومجموعة الاضلام كانت لديهما تحسن فيعدل التنفس ودرجة حرارة الجسم والاستجابة المناعية مقارنة بالمجموعة الكنترول. لا توجد اختلافات معنوية بين مجاميع المعاملات والكونترول في مستوى هرمون  $IL_1$  وقيم الكرياتينين وحامض اليوريك في مجموعة المنع الغذائي و مجموعة الاضلام انخفضت معنويا مقارنة بالمجموعة الكنترول لا توجد اختلافات معنوية بين مجاميع المعاملات والكونترول في مستوى بروتينات والبيومين والجلوبيولين في البلازما .

ومما سبق فإن الدراسة أوضحت في مجملها أن فترة منع التغذية او الاضلام اثناء درجات حرارة البيئة العالية على الاداء الانتاجي للبط المسكوفي تسببت في خفض حرارة الجسم وتحسن بعض الاستجابات الفسيولوجية والمناعية البط خلال فترة الصيف. لذا ليس هناك مانع من استخدام تلك الطرق لمواجهة الآثار السلبية لارتفاع درجات الحرارة.