الآية

صدق الله العظيم

سورة طــه : الآية(114)

Dedication

This dissertation is dedicated to our parents whose affection, love, encouragement , and prays of day and night make us able to get such success and honor, as well as our sisters, brothers, all who we love them and our supervisor Dr. Selma El Malieh for her unwavering support, collegiality , and mentorship throughout this project.

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Abbreviations

EDTA: Ethylene diamine tetra acetate.
CBC: Complete blood cell count.
SPSS: Statistical package for social science.
RBCs: Total red blood cell count.
HGB/Hb: Hemoglobin concentration.
HCT: Hematocrit.
PCV: Packed cell volume.
WBCs: Total leukocytes count.
BW: Body weight.
SD: Standard deviation
ESR: Erythrocyte sedimentation rate.

Abstract

The study were carried out in poultry production unit (college of science and Technology of animal production) during June, 2017 to investigate the effects of heat stress, hungry stress, overcrowding stress and thirst stress on physiological responses in Leg horn broilers chicken. Two hundred and fifty, one-day-old male, broiler chicks were used in this study, The chickens were randomly divided into five treatment groups for 30days fifty of each to investigate the effect of heat stress, hungry, overcrowding and thirst. The last fifth saved as a control group. The birds were fed on per starter and starter diet, 2 ml of blood were taken from the heart in EDTA anticoagulant container ,Standard method was used to measure hematological parameters such as:(PCV, Hb, ESR, TWBCs and TRBC s) and body weight .All the values were calculated as the mean± standard deviation while the difference between the means was compared by an independent student's test using statistical package for social science (SPSS version 16). The results indicated that heat stress group had significantly ($P \geq 0.01$) lower PCVand BW and significantly($P \geq 0.05$) lower Hb concentrations, TLC compared to the control group in chickens. Hungry stress in chickens indicated significantly($P \geq 0.01$) lower PCV,Hb concentration, TRBC and BW and higher TLC compared to control group. Overcrowding stress in chickens indicated numorous decrease in PCV and Hb concentration ,TRBC, TLC. But BW values decrease significantly ($P \geq 0.05$) in overcrowding chicken. The thirst stress chickens had higher PCV, Hb concentration, TRBC, TLC ($P \geq 0.05$) and lower BW values($P \geq 0.05$) compared to control group.

Further research is required to fully understand interaction between food restriction, body weight, health and fertility. And determine when and how much food restriction

المستخلص

اجريت الدراسة في وحدة إنتاج الدواجن(كليةالعلوم وتكنولوجيا الإنتاج الحيواني) خلال يونيو/2017 للتحقيق اثار الإجهاد الحراري ،الجوع،الإزدحام والعطش،على لإستجابة الفيسولوجية في الدجاج leghorn

استخدمت مائتان وخمسون دجاج اللاحم ذكور عمر يوم تقسيم الدجاج عشوائيا لخمسة علاج لمدة 30يوم خمسين لكل منها للتحقيق في تأثير اثار الإجهاد الحراري ،الجوع،الإزدحام والعطش واحر قروب تم حفظه كمجموعة تحكم تم تغذية الطيور علي علف قبل البادئ والبادئ .تم اخذ 2مل من الدم مباشرة من القلب في انبوب يحتوي علي مضاد تجلط بإستحدام الطريقه القياسية لمقاييس الدم (تركيز هيموجلوبين، مكداس الدم، العدد الكلي لكريات الدم الحمراء، العدد الكلي لكريات الدم البيضاء ووزن الجسم.تم حساب كل الأوساط±الإنحراف المعياري بينما الإختلاف بين الاوساط تم مقارنتها بإستخدام الحزمة الإحصائية النسخة16 اظهرت النتائج إن الضغط الحراره في الدجاج انخفض تركيز هيموجلوبين، مكداس الدم، العدد الكلي لكريات الدم الحمراء، العدد الكلي لكريات الدم البيضاء مقارنة مع مجموعة السيطرة’ .الجوع في الدجاج ظهرت انخفاض تركيز هيموجلوبين، مكداس الدم، العدد الكلي لكريات الدم الحمراء وزيادة في كريات الدم البيضاء مقارنة مع مجموعة السيطرة’الإزدحام في الدجاج ظهرت انخفض تركيز هيموجلوبين، مكداس الدم، العدد الكلي لكريات الدم الحمراء، العدد الكلي لكريات الدم البيضاء مقارنة مع مجموعة السيطرة أما العطش في الدجاج ظهرت ارتفاع في تركيز هيموجلوبين، مكداس الدم، العدد الكلي لكريات الدم الحمراء وزيادة في كريات الدم البيضاء مقارنة مع مجموعة السيطرة. وظهرت انخفاض وزن الجسم في كل من الضغط الحراري’الجوع’العطش والإزدحام. مطلوب مزيد من البجوث لفهم كامل التفاعل بين الجوع’ وزن الجسم ,الصحة والخصوبة .وتحديد زمن وكيفية القيود الغذائيه

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INTRODUCTION

Sudan is large country in Africa covers an area about 2-5milion km^2 comprised of desert and air land which change temperature and rainfall is not continuous and differ from north to south it is occupies in tropical zones between $3^o$and 23^onand 21^o and 36^oE ,mean annual temperature lies between 26^o to 32^o but in some areas it is reach 47c ^{o} causing stresses and heat related daises'(Hcenr,2003). Poultry are generally birds of indigenous breed living in relationship in the human communities (Spradb Row,1993) out of total population of 45,3 million chicken in Sudan, the traditional sector comprises around from meat and egg production (Suliman,1996) .

Poultry body temperature is 41.5c^o but will fluctuate somewhat depend upon temperature of its environment (Sottink ,2002) The indigenous breed include large Baladi, Bar-neck and Betwil (Desal,1962). The common system type of poultry Sudan include closed system which using air condition ventilation fans to regulate weather inside the houses, modern closed system which in large companies under controlled environment .Battery cages which hens confined to cage just large enough to limited movement and standard sit comfortably, open system which is ventilated cool in summer, warm during winter and free from drafts.(Appleby,Michaelc.1992)

Chicken are waste coverts, convert to feed resource base in to animal protein (Eekeren *et al.*,2006) poultry have high economic value products are cheap in price compeers' with other types of animal protein (Baabound and Hayat.,1981).Poultry raring management is regarded major determination of health status ,factor influenced the bird health and welfare including house structure ,climate condition ventilation temperature stocking density ,feed and water supply hygienic condition (Charata *et al*, 2012).

Physiological indicator of welfare include the measurement of the main regulatory function such as heart rate ,rectal temperature, hormones and blood constituent in addition to behavior indicator such as panic and escape however changes in this parameters may vary depending upon the nature of stressor

Research objectives

General objectives:

The main aim of this study was to investigate changes occur in body weight and Hematological parameters during stresses of Heat, Thirst, Hungry and Overcrowding in chicken

Specific objectives**:**

To measure:

1. The change in body weight during stresses
2. To determine the effects of stresses (heat,thirst,hungry,overcrowding) on erythrocyte series(PCV, Hb , ESR, and total erythrocyte count)
3. To evaluate the effects of stresses(heat, thirst, hungry, overcrowding) on total leukocyte count

Chapter one

Literature Review

Chapter one

Literature Review

1.1 birds scientific classification

- Kingdom : Animalia
- Phylum: chordate
- Class: Aves
- Order: Galliformes
- Family : Phasianidae
- Subfamily: Phasianinae
- Genus : Gallus
- Species : G.gallus
- Subspecies: G.g.domesticus

Stress is the nonspecific response of the body to any demand, its represents the reaction of the animal organism to stimuli that disturb its normal physiological equilibrium or homeostasis(Selye,1976).Animal welfare is completed freedom, if there are lake of hunger and thirst, lack of discomfort, pain, injury, and lake of fear and depression. Welfare of bird is to large extend regulate by various intrinsic and extrinsic (Ravidndran *et al.*,2006)

1.2 Different stresses in chickens

1.2.1 Hormonal Responses

In chicken cortisol is the main hormone associated with stress.Experimental administration of corticosteroid is use to induce stress it concentration rises in blood under stressful condition.changes in corticosterone may have other effects on hormonal system, such as the conversion of nor adrenaline into adrenaline or production of thyroid hormone .elevation of corticosterone also have immunosuppression effect.

1.2.2 Enzymes responses

Some plasma enzyme have been used as indicator of stress include creatinine kinase (CK) and alkaline phosphate (ALP),aspartate transaminase(AST) lactate dehydrogenase(LDH).the rise in concentration in enzyme reflect tissue damage (Hocking et al.,1993) the authors added that ALP can release from liver and muscle and is a good indicator of hemolysis, but CK is particularly good indicator of heat stress

1.2.3 Behavior responses

Several behaviors have been associated with stress fearfulness induced by severe stress(Jones and faure1981).. Other fear reaction include panic and violent and escape. Restricted birds show an increase spot pecking, scratching and preening(Hocking *et al.*,1996). The author pointed that increase drinking it also behavior response to feed restriction. Panting is good indication in birds under stress

1.2.4 Heat stress

Heat stress is one of the most important environmental stressors challenging poultry production in temperate and tropical regions of the world(Balnave, 2004).Under high temperature condition bird alter their behavior and physiological hemostasis seeking thermoregulation in general; different types of birds react similarly to heat stress. Heat stress is condition caused by high environmental temperature and relative humidity which reduced heat loss and increase core body temperature(CBT)(Jensen and Toates,1997).An increase of CBT by 4^{o}c leads to death in bird (Defra,2005).Heat stress affects negatively on growth rate, meat quality egg production and immune function and also can lead to higher motility(Quinteiro-filho *et al* 2010).This effects can decrease the sustainability of commercial chicken production and have severe economic implication for broiler industry (St-pierre *et al.*,2003).

A recent study showed that bird subjected to heat stress condition spend less time feeding more time drinking and panting as well as more time were there wings elevated less time moving and walking and more time resting (Mack,*et al*., 2012).heat stress also causes reduction on feed intake and increase water intake (May *et al* 1997). In a study combined measures of feed intake during heat stress(32^{o}c), there was reduction in rate of eating with in meals in domestic laying fowls (Rhod island Red×Light Sussex) compared to those kept in normal condition (20oc) (Savory,1986).

Under tropical conditions, fowls are exposed to marked thermal stress. The heat load imposes severe stress and results in reduce physiological and reproductive performance (Abdalla and Nawal, 2009). Stress in poultry can decrease erythrocyte count(Toghyani *et al*., 2006),hemoglobin concentration (Hilman *et al*., 2000; Puvadolpirod and Thaxton, 2000),percentages of leucocytes(Nathan *et al*.,1976; Mashaly *et al*.,2004),packed cell volume (PCV) values varies with environmental temperature .A decrease in PCV values has been absorbed in broiler chicken exposed to 24hr of 30c(Yahava and hurwitz.,1996)

1.2.5 Thirst stress

Thirst is the subjective perception that motivates an animal to drink ,is considered to have a major impact on animal welfare (Vanhonacker *et al*.,2008; Tuyttens *et al*., 2010). Thirst caused dehydration which reduce production performance (Springer *et al*.,2009).High stocking density of poultry house can make it move difficult for animal to access water(Feddes *et al*,2002).described that prolong thirst cause stress and it long lasting or severe lead to depletion ,loss of body condition and disease.(JONES *et al*, 2009).

Thirst is activated by several factors related to water loss or dehydration {e.g., decreased bodily fluids, increased blood osmolality, or changes the activity of specific dipsogenic hormones (Mc kinley and Johnson, 2004). Several these physiological changes have shown potential as indicators of thirst (Vanderhasselt *et al*.,2013). However, physiological mechanisms buffer fluctuations in blood parameters (Springer *et al* .,2009).

1.2.6 Overcrowding stress

Most of today problems in poultry are caused combinations of factors such as management, stress, feeding, overcrowding, poor ventilation (Isohe and Lillehoj,1992).Overcrowding of broiler chicks due to lack of space induces harmful effects which act as predisposing factor in the reduction of production and performance in poultry and immunosuppression (Sexena and Madan, 1997) and disrupts physiological and psychological stability of chicken and reaction of stressor (Pande,2002).

The overcrowding effect on welfare and performance and immune status of birds and body Wight gain and feed consumption were adversely affected by incense the housing density.(Dozier *et.al* 2005). There was a reduction of feed intake in response to increasing density even though the feeder space per bird was kept constant (Scholtyssek and Gschwindt- Ensinger, 1983), and in some cases the depression of feed intake was higher than the reduction of growth rate. This resulted in a better feed conversion in overcrowded birds(Scholtyssek and Gschwindt-Ensinger, 1980).

A mild feed restriction may be the cause for reduced feed intake with increasing stocking density and feed conversion may become worse as heat stress further increases and/or ammonia level rises (Johnson *et al.*, 1991).The interrelationships between stocking density and pathologies, such as chronic dermatitis, breast blisters and leg disorders have been reported in various experiments. Pattison (1992) stated that stocking density is the main husbandry factor which increases the level of mortality resulting from leg disorders.

1.2.7 Hungry stress:

Feeding is amongst the most natural of all animal behaviors as it has a profound effect on the survival of the individual and its reproductive success, i.e. its evolutionary fitness. Normal feeding behavior of an animal with continuous access to food shows a typical temporal structure of feeding bouts (frequently called meals) separated by non feeding intervals (Tolkamp *et al*. 2011).

Feed restriction leads to stress resulting from hunger and frustration, which may result in impaired health and welfare. Fluctuating asymmetry (small random deviations from right-left symmetry in bilateral traits) has been reported to reflect well-being status and chronic stress (Mollar and Swaddle, 1997).

Hematological parameters in chickens have shown to be influenced by nutrition packed Cell Volume (PCV), Hemoglobin (Hb) concentration and Red

Blood Cell (RBC) counts have been reported to increase with age (Islam *et al*, 2004).

1.3 Hematological parameters

The values of blood constituents are affected by many factor s such as physiological condition , environmental and diet and management, also sample collection and method of laboratory analysis play important role. Hematological change have been used to assess the level of stresses due to various physiological situation (Khan and Zafar,2005).

The avian CBC is most important component of diagnostic panel and the best indicator of bird general health. The hematological parameters of health birds are influenced by many factors which include physiological, physical ,and environmental conditions, fasting and nutrient conditions(Alodan *et al*., 2004) environmental factors (Vecerek *et al*.,2002; Graczyk *et al*.,2003) fasting(Lamosova *et al*., 2004) age (Furlan *et al*.,1999; Naziefy, 1997; Seiser *et al* 2000 ; Talebi *et al*., 2005)

1.3.1 Red blood cells count

Red blood cells count (Erthrocyte) is the number of red blood cells per cubic millimeter of blood.it is measured by using microscope and a ruled chamber called neubar hemacytometer and the number of RBC is in millions per cubic millimeters(Hamouda *et al*.,2012). The red blood cells are among the cellular part of the blood which are biconcave in shape and transport oxygen and carbon dioxide from one part of the body to another (Guyton and Hall.,2006). They are synthesized in the bone marrow (Gannog,2003).

Depressed bone marrow development results in decreased production of the red blood cells and red blood cells count (Coles,1986).A significant difference in the number of red blood cells exist between the individuals of the same breed due to factors such as sex, age, physical activity, nutritional status, laying cycle, altitude, stress and management (Sjaastad *et al*.,2003;coles,1986).

1.3.2 Packed cell volume

Centrifugation of the heparinized blood sample separates blood cells from the plasma, red blood samples are found packed at the bottom of the centrifuge tube while the leucocytes, blood platelets appearing as a thin grayish layer above the erythrocyte, This separation of red blood cells and other cell component is due to differences in mass density (Sjaastad *et al*., 2003).

Packed cell volume PCV(%) is one of the indicators of high feed conversion efficiency in chickens (Mmereole,2004). MHhonzya,(2002) reported that hemoglobin and packed cell volume levels are normally higher in a well nourished then poorly fed animal. PCV(%) is erroneously decreased in blood sample with excessive EDTA or marked hemolysis. Packed cell volume decreases when chickens are exposed to heat stress (yahav *et al*.,1997). Packed cell volume has been accepted as indicator for heat stress in chickens (Aengwanich,2007).

1.3.3 Erythrocyte sedimentation rate

If the uncoagulated blood is kept in Erythrocyte Sedimentation Rate westegreen tubes and left for sometimes without being disturbed, the blood cells settle at the bottom of the ESR standard tubes and pale yellow plasma is observed on top of it . this is due to the differences in mass density, the tendency of the red blood cells to cluster together and composition changes in plasma which increases aggregation of red blood cells in the diseased animals as well as nutritionally deficiency in proteins and vitamins especially vitamin B group (Sjaasted *et al.*, 2003) . If there is an abnormal RBCs such as sickle cell, clustering together of RBCs is hindered and hence ESR is decreased (Jandl,1996).

1.3.4 Hemoglobin concentration

Hemoglobin synthesis starts in the pro-ethroblasts and goes on in the reticulocyte of red blood cells . the hemoglobin combines with oxygen in the lungs and releases oxygen in the areas with low oxygen tension ,(Guyton and Hall.,2006). Hemoglobin concentration together with packed cell volume and red blood cell count determine the red blood cells indices which are MCH(pg) and MCHC(%) (Elagib *et al*., 2011)

Hemoglobin and myoglobin (in iron and oxygen binding protein found in the muscle tissue of vertebrates) are important parameters which indicate meat quality, they effect the color of meat and can cause unsuitable discoloration when they exudate from muscle tissue or extravasate from the circulatory system of the chicken (kranen *et al*., 1999) .

1.3.5 White blood cells count

White blood cells are among the blood cells synthesized in the stem cell of the bone marrow. They are sometimes called leukocytes. They are five types of leukocytes, which are neutrophils,eosinophils,basophils,monocytes and lymphocytes. The first three are classified as granulocytes and the last two are classified as agranulocyte (Sjaasted *et al.*, 2003)

White blood cells are transported to areas of serious infection and inflammation, thereby providing a rapid and potent defense against infectious agents (Gutyon and Hall, 2003) the granulocyte and monocytes (agranulocytes) have a special ability to “seek out and destroy “a foreign invader (Guyton and Hall ,2003) white blood cell count are affected when the layer chicken are subject to excessive heat stress (Mashaly *et al*.,2004) white blood cells carry out their function by producing antibodies which deactivate the foreign substances known as antigen . An antigen is an agent or particle which stimulate the production of specific antibodies, cell-mediated immunity, or both (Ferguson, 1985).

The antibodies are serum or cell-bound proteins produced to an antigen and able to react specifically to that antigen. Some white blood cells (monocytes) have the ability to engulf the foreign particles by phagocytosis (Ferguson, 1985).The main Haematological response to stress is the neutrophil /Lymphocyte ratio (H/L) in leukocyte . The number of neuterophil s increases and the number of lymphocyte decreases in bird under stress (Gross and Siegel, 1983).The changes in H/L have been observed in responses to thermal and prolonged feed restriction (Maxwell *et al*, 1991). Basophils can also be increased during stress.

Table 1: Normal blood values for chickens (Galusgalusdomesticus)

Blood parameter	Range	Average
RBCs (x 10^6/ µl)	2.5-3.5	3.0
Hemoglobin (g %)	7.0 - 13.0	9.0
PCV (%)	22.0 -35.0	30.0
MCH (Pg)	33.0 – 47.0	41.0
MCHC (%)	26.0 -35.0	29.0
ESR (mm)	3.0 -12.0	7.0
White blood cells (x10^4/)	1.2 -3.0	2.1

Source; Jain, N.C (1986)

Chapter Two

Materials and Methods

Chapter Two

Materials and Methods

2.1Date and place

The study was performed to evaluate the effect some environmental stresses on hematological responses of chicken. The study was performed in June 2017,in the Poultry Production Unit of Department of Animal Science and Production(DASP),Sudan University Science and Technology (SUST) altitude 2*3mm area of poultry barn

2.2Experimental birds and management:

The total number of birds was two hundred and fifty one day old white leghorn cocks, Farn was sterilized with hawasan and formalin10% the weight of the birds in the first day was 50-60g , the chicken were examined clinically and were kept the bran for an adaptation period of 5-7days followed by an experimental period for 4weeks the weeks of the adaptation the birds were fed commercial available mixture with per starter and recommended with neoxyvital (oxytetracycline,neomycin,vitamins,minerals) for prevention.The chickens were randomly divided for fivetreatment groups for 30days fifty of each . The five treatments groups one group was saved as control ,group as heat stress, group as hungry stress, group as thirst stress,group as overcrowding stress fed the birds with per- starter and starter for broilers the duration of the experimental enough to note the effect of the stresses in the hematological parameters of chickens

2.3Experimentalgroups

2.3.1 Heat stress:

Four lambs 100watt used for this stress the time exposure to light is 8AM-7PM per day the normal temp was 38-39c^o, the temperature exposure was 40-42c^o.

2.3.2 Thirst stress

This stress stop the drinking to every two hours per day that mean was fasting in the waterfor 2hrs then drinking and remove the water rapidly this way was used for 4times per day

2.3.3 Hungry stress

This stress fasting for every 4hours then eating and rapidly removed the silage in this way was used these ways for 2times daily

2.3.4Overcrowding stress

The normal adequacy area for one chick is10cm and this stress used to narrowing of the space area of the one chick from10cm to 1cm.

2.3.5Control group

This group has adequacy water and silage until one month and enough area for chickens.

2.4 Experimental parameters

2.4.1 Body weight

The chicken were weighting by using digital balance (Omega),China.

2.4.2 Blood Parameters:

The hemoglobin concentration (HB),packed cell volume(pcv),Erythrocyte sedimentation (ESR),and total leukocyte count were determined according to standard method as described byJain (1993)

2.4.3Sample collection:

Ten growers were picked up randomly for each treatment group for blood sample collection and every ten treatment group was picked up ten of keeping group(control).2 ml of blood were aseptically collected using a sterile needle and syringe of five millimeters from the heart puncture of chicken. Immediately after collection the blood was transferred for tubes containing anticoagulant Na_2(EDTA)to prevent it from clottingThe blood collected were analyze at the Department of Biomedical Sciences and Technology in the faculty of veterinary medicine, Sudan university science and technology, Helat Koko, Khartoum, Sudan so as to get the hematological parameters

2.4.3.1Hemoglobin concentration:

The hemoglobin concentration in the blood samples use were determined in Sahli apparatus method the graduated Sahli tube was filled with 10%HCL up to the graduated mark on the scale and the blood was sucked using capillary pipette up to the 20µ mark excess blood was removed by cleaning the tip of the pipette using filter paper. Then the blood was puffed for the pipette 10%HCL in the graduated Sahli tubes keeping the tip in the HCL solution. Inflated and sucking using pipette for several times to make sure all blood has been removed from the pipette .the color change, dark brown of the mixture was determined same drops of distilled water was added in to mixture until the color become similar to the standard solution was obtained finally the graduated sahli tube was removed and read in g/dl

2.4.3.2Packed cell volume:

To gain the PCV a well-mixed blood sample in the tube was filled in capillary tubes at three quarter level, and the seal at one end by clay. The sealed capillary tube were transferred in the hematocrit centrifuge machine was made in china use 1500round per 5minutes then the centrifuged capillary tubes were taken out of the machine and the sediment blood were read for hematocrit ruler in percentage

2.4.3.3Red blood cell count:

In assisting the RBC count,0.5 mark of the blood samples using a thoma glass pipette with red glass bead inside, and mixed with diluting fluid until the mark of 101 (hayem’s fluid) is isotonic with blood it is composition is(Nacl ,$Na_2so_,Hgcl_2$and distilled water).contents were mixed carefully shaking for two minutes then the blood were place in clean dry neubauer chamber of hemacytometer with cover slip to be sure the space between the cover glass and the ruled area without excess. The filled counting chamber was left for 3minutes for cells to set in the chamber. using light microscope×40 objective ,red blood cells were manually counted in the four corners and central square R in chamber, this area is subdivided ,which in turn are each divided in to 16squares. Of the 25medium only the four corner squares and the center square of R

Calculation of red blood cells:

$$\text{Total RBC/mm}^3 = \frac{\text{NUMBER OF RBC COUNTED} \times \text{ DILUTION FACTOR} \times \text{DEPTH FACTOR}}{\text{NO OF CHAMPERS COUNTED}}$$

Result:

Total RBC count of the given blood sample ____milion/mm^3

2.4.3.4 Erythrocytes sedimentation rate:

The un coagulated blood samples were taken and put in the westegreen tubes until 0 mark then The ESR westegreen tubes containing the blood sample were placed in the sedimentation rack vertically were read in mm after been left 1hour

2.4.3.5White blood cells count:

To assess the total white blood cell,20µl of blood sample was taken up to 0.5 mark with thoma pipette witch has a white glass bead and mixed with turck's fluid is composed (acetic acid,gelatin violet or methylene blue)up to 11 mark and mixing for minute some method using for RBC the mixture was filled into clean dry neubauer haemocytometer slide with cover slip and then show by using light microscope×10 objective, WBC were manually countedthe for W corner squares of the both champers were counted

$$\text{Total WBC/mm}^3 = \frac{\text{number of WBCcounted} \times \text{dilutionfactor} \times \text{depth factor}}{\text{No of champers counted}}$$

Result :

Total WBC count of the given blood___/ mm^3

2.5 Statistical analysis:

The experimental data were collected and subjected to analysis of T-test using the SPSS package to evaluate the effect of stresses on hematological parameters as thirst,heat,hungry and overcrowding the values of parameters measured are expressed as means ± standard deviation.

Chapter Three

Results

CHAPTER THREE

Results

3.1 Effect of the heat stress on some hematological parameters on chicken and body weight indices:

Table (2) shows that the PCV values(%) of control and treated chicken groups .The values of PCV significantly (P<0001) in heat stressed groups. heat stressed group had significantly (p>0.05) lower Hb values compared with control group. There was numerous decrease of RBC and ESR attributed to effect of heat stress. The effect of heat stress on WBCs shows lower significant ratios in stressed group. The effect of heat stress on body weight (B.W) has been shown in table 3-1. The B.W of heat stressed group (154g) was significantly (p≤0.01)lower than in control group (170g).

3.2 Effect of hungry stress on some hematological parameters and in body weight indices:

Table (3) shows that the PCV ,Hb, TRBC values decreased significantly in bird subjected to hungry compared to control group. But TLC increased significantly (p<0.01) in treated group. The stressed group shows marked decrease (p<0.05) in body weight compared to control group . however, significant change in ESR values associated with heat stress .

3.3 Effect of the overcrowding stress on some hematological parameters and body weight inthe chicken indices:

Table (4) shows that HB concentration, PCV values ,TRBC and ESR of chicken group showed slight decrease in overcrowding group .The total WBCs shows numerous increase in treated group than in control group.But, BW of treated group decreased significantly(p<0.05).

3.4 Effect of the thirst stress on the some hematological parameters and body weight in the chicken indices:

Table(5) Indicates Hb concentration ,PCV values, RBCs,WBCs had showed significant (p<0.05) increase in thirst group compared to control group but the ESR values shows non-significant decrease in thirst group compared to control group. BW decreased significantly($p<0.05$) in thirst group compared to control chicken.

Table3.1 Effect of heat stress on some hematological parameters and body weight in chicken

Parameters Groups	Control	Heat stress	Sig. level
Hb(g/dl)	6.95 ± 0.5	6.30 ± 0.30	*
PCV(%)	22.6 ± 4.45	15.9 ± 1.9	**
WBCcount($*10^3$/ml)	6.8± 0.04	5.6 ± 0.1	*
RBCcount	4.7 ± 1.7	4.5 ±2.9	N.S
ESR(mm)	10.1 ± 4.6	8.7 ± 4.5	N.S
B.Wt (g)	170.6 ± 14.43	154 ± 8.11	**

* = Significant level at $p\leq0.05$

** = Significant level at $p\leq0.01$

N.S = non significant.

Table3.2 Effect of hungry stress on some hematological parameters and body weight in chicken

Parameters Groups	Control	Hungry stress	Sig.level
Hb(g/dl)	7.3 ±1.14	6.6 ± 0.74	**
PCV(%)	27.1 ±4.1	23.3 ± 1.6	**
WBCcount(*10^3/ml)	1.81 ± 0.65	3.18 ± 0.45	**
RBC count(*10^6/ml)	2.94± 0.40	2.12±0.41	**
ESR(mm)	3.2± 0.781	3.1±0.788	N.S
B.W (g)	155 ± 9.5	124 ±17.3	*

* = Significant level at p≤0.05

** = Significant level at p≤0.01

N.S = non significant.

Table3.3 Effect of overcrowding stress on some hematological parameters and body weight in chicken

Parameters Groups	Control	Overcrowding stress	Sig. level
Hb(g/dl)	8.2 ±0.87	7.2 ± 0.72	*
PCV(%)	33 ± 3.19	30.50 ± 2.95	*
WBCcount($*10^3$/ml)	2.4±0.54	3.01± 0.7	N.S
RBC($*10^6$/ml)	2.83±0.39	2.78±0.51	N.S
ESR(mm)	3.4 ± 1.34	2.4 ± 0.48	N.S
B.Wt(g)	142 ± 13.6	120 ± 15.4	*

* = Significant level at $p \leq 0.05$

** = Significant level at $p \leq 0.01$

N.S = non significant.

Table 3.4 Effect of thirst stress on the some hematological parameters and body weight in the chicken

Parameters / Groups	Control	Thirst stress	Sig.level
Hb(g/dl)	8 ± 0.69	9.7 ± 1	*
PCV(%)	24.7 ± 1.8	29 ± 3.33	*
WBCcount($*10^3$/ml)	4.29± 0.55	6.1± 1.8	*
RBC($*10^6$/ml)	3.2±0.8	4.11 ± 0.4	*
ESR(mm)	5.7 ± 2.7	4.3 ± 1.4	N.S
B.Wt(g)	162 ± 13.7	132 ± 14.7	*

* = Significant level at $p \leq 0.05$

** = Significant level at $p \leq 0.01$

N.S = non significant.

Chapter FOUR

Discussion

Chapter four

Discussion

The study was done to assess the effect of heat stress, hunger, thirst and overcrowding on hematological parameters and body weight of growing layer chickens was done.Hematological parameters(PCV, Hbconcentration, WBC,TRBC and ESR) and BW were determined in the study

The present results confirm that there was significant decrease in PCV values in leghorn chickens during heat stress, high ambient temperature exposure inhibits the production of erythrocyte in the bone marrow (Puvadolpirod and Thaxton,2000;Toghyani *et al,* 2006). The decrease of PCV was caused by the high ambient temperature exposure that lead to the decreased number of erythrocytes (erythropoiesis). The decrease of hematocrit value might be due to the damage of erythrocytes, the decrease of erythrocyte production or the decrease of erythrocytes number and size (Coles, 1982; Hilman *et al.,* 2000; Altan *et al*., 2000) or due to the increase in water consumption during the exposure to heat stress (Tamzil *et al*., 2013) that eventually diluted blood cell concentrations, included leukocyte. The results are in agreement with previous study who reported that acute heat stress in poultry lowers of erythrocyte, hemoglobin, hemoctrict,and leukocyte (Noor and Seminar, 2009)

The present results showed that there was significant decrease in Hb concentration in leghorn chicken attributed to heat stress, this effect may related higher environmental temperature on changes in distribution of iron in the organism of broiler (Jamadar and Jalnapurkar 1995).The decrease in Hb concentration agrees with (Comito etal., 2007 ;Awad ,2012)

The current result demonstrated that in chickens there was adecrease in total red blood cells. During heat stress this effect might be due to decrease in oxygen consumption by the chicks as result of high ambient temperature (khan et al .,2002). Huston et al. (1962) reported a of reduction hempoetic activity attributed to high ambient temperature.The present result is agreement with previous study by (Faisal ,2008 ; Awad, 2012)

WBC decreased during heat stress. exposure of broilers to stress releases glucocorticoid causing dissolution of lymphocytes in lymphoid tissue and lead to lymphopenia and increase hetrophils which increase the hetrophil/lymphocyte ratio (Gross and Siegel,1983) several studies have been investigated the effect of heat stress on immune system responses of broilers decrease of lymphocyte and increase og H/L ratio during heat stress have been reported by Bednovea *et al*.(2006)and Altan *et al*.(2000).

The results from Table (2) indicated that bodyweight values was decreased in heat stressed group.This might be associated with decrease feed intake.High ambient temperature reduce the thyroid function and consequently, metabolic rate oxygen consumption and growth rate (Moraes *et al* 2003). The results agrees with Zuprizal *et al*.(1993) who reported decreased BW during heat stress attributed the reduction in body weight gain during heat stress episode to the reduction in both feed consumption and true digestibility of protein and amino acids. .Cooper and Wash-burn (1998) reported that broilers BW was significantly greater in the 21^{o}c environment than in 32^{o}c.

There was a significant decrease ($p<0.05$) of hungry on PCV and Hb concentration and TRBCs in broilers(Table 3).The results could be associated with fasting period which decrease the feed intake during the experiment. Maxwell *et al*.(1992b) studied the effect of feed restriction on erythrocyte series and reported a significant change in PCV, Hb concentration ,TRBCs ,TWBCs but Junqueira et al (2003) found no effect of feed restriction on PCV,Hb,TWBCs and RBCs. The current results indicates that BW decreased in broilers subjected to hungry .The effect may be associated with decreased feed intake conversely (Yu and Robinson, 1992) reported that the body weight of broilers was not effected by feeding restriction in 2hrs at a time

The present result in Table 4 indicate that overcrowding decreases PCV,Hb concentrations, TRBC,ESR significantly .A reduction in PCV with increased stocking densities from 24 to 32kg /m^2 has been reported in broilers (Scholtyssek and Jschwindt-Ensinger,1980) but the authors (Scholtyssek and Jschwindt-Ensinger,1983) could not confirm previous result for PCV under density from 25to39 kg/$m^{2.}$ But some studies indicate that overcrowding elevated Hb concentrations , causes by less supply of oxygen to bird and hypoxia ,which stimulate erythropoitensecreation and lead to erythropiosis ,(Bedanova, 2006 ; Lokhanda *et al*.,2009)

The present result suggested that overcrowding did not affected significantly on TLC values. Previous studies reported reduction of TLC values (Saxena and Madan,1997 ;Rizketal.,2003 and Rosales *et al*,1994) Karthiayini and Philomina (2008) reported that overcrowding did not affected on Hb concentration .

The current result in table 4 indicate that BW decreases in overcrowding group. There was reduction of feed intake in response to increasing density (Scholtyssek and Jschwindt-Ensinger,1983) Singh and Sharma (2003)reported depression in BW in bird exposed to overcrowding, they revealed that overcrowding result in poor growth. The result is agree with Rizk *et al*. (2003) and Thomas *et al*. (2004). Lokhande *et al*.(2009) observed that overcrowding causes reduction in BW in bird groups provided space 25% and 50% less space

The result in Table 5 indicate that thirst increases the PCV , Hb concentrations and $TRBC_S$ values in birds. The effects may be attributed to decrease of blood or plasma fluids in thirst groups. Woerpel and Rosskopf(1984) observed increase in PCV with severity of water restriction suggested a depletion of plasma water that consequently reduce plasma volume leading to heamoconcentration.An increase of PCV during thirst period has been reported by (Vanderhassel,2013)in broilers chicken after 6hrs of thirst .Conversely ,Pires etal .(2007) and Iheukwumere and Herbert (2003) observed reduction in erythrocyte count of water restriction broilers

The current result investigate TLC increase in thirst broilers .The findings agrees with Maxwell *et al*., (1992) ,who showed that immune status of bird change with intensity and type of stress to which they are exposed . Nutritional and stress include leukocytosis and lymphopenia (Maxwell 1993).

The results in table 5 also indicate significant decreased body weight as result of thirst effects .The decrease of water intake may be attributed to decrease in feed intake and body fluids .Vanderhassel (2013) revealed that thirst of 24 hours causes decrease in body weight of broiler chicken .

CONCLUSION

Based on the results of this study, the following conclusion and recommendations were drawn:

Conclusion:

1/ There was heat stress effect on hematological parameters and body weight . Heat stress caused a decrease in PCV, Hb,TRBC, TLC, and BW.

2/ The effects of hungry on physiological responses broilers

been assessed. The chicken exposed to hungry had lower PCV, Hb concentrations, TRBC and BW and higher TLC compared to control group.

3/ The effect of overcrowding on hematological responses and BW were investigated in chicken. The overcrowding group maintained lower PCV and Hb concentration ,TRBC, TLC and BW was reported in overcrowding chicken.

4/ The effect of thirst on physiological Reponses were evaluated in broilers. The thirst group had higher PCV, Hb concentration, TRBC, TLC and lower BW compared to control group.

Recommendations

1/ A range of behavioral and physiological changes has been used to identify and quantify stress. These changes may differ qualitatively or quantitatively depending on the stressor so that a range of indices should be used in order to assess the extent of the stress or welfare.

2/ Severe restricted diet and heat stress are associated with the stressfulness of chicks, and additives dietary supplementation is effective to alleviate the Stress. Good nutrition is important for broilers healthy and might improve bird health and welfare.

3/ Nutritional strategies and appropriate husbandry practices could be implemented to alleviate heat stress and improve productivity . Nutritional management can have an impact on metabolic disorders.

4/ Water restriction reduces food intake, which in turn may cause hunger.

5/ There is a clear tendency for reduced growth rate at high stocking densities in broilers. The negative effects of overcrowding on growth rate are reduced when adequate ventilation rates are provided. This indicates that problems of heat dissipation are the main causes of poor growth of overcrowded broilers.

6/ More research is required to fully understand interactions between food restriction, body weight, health and fertility, to determine when and how much food restriction is required.

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Appendix


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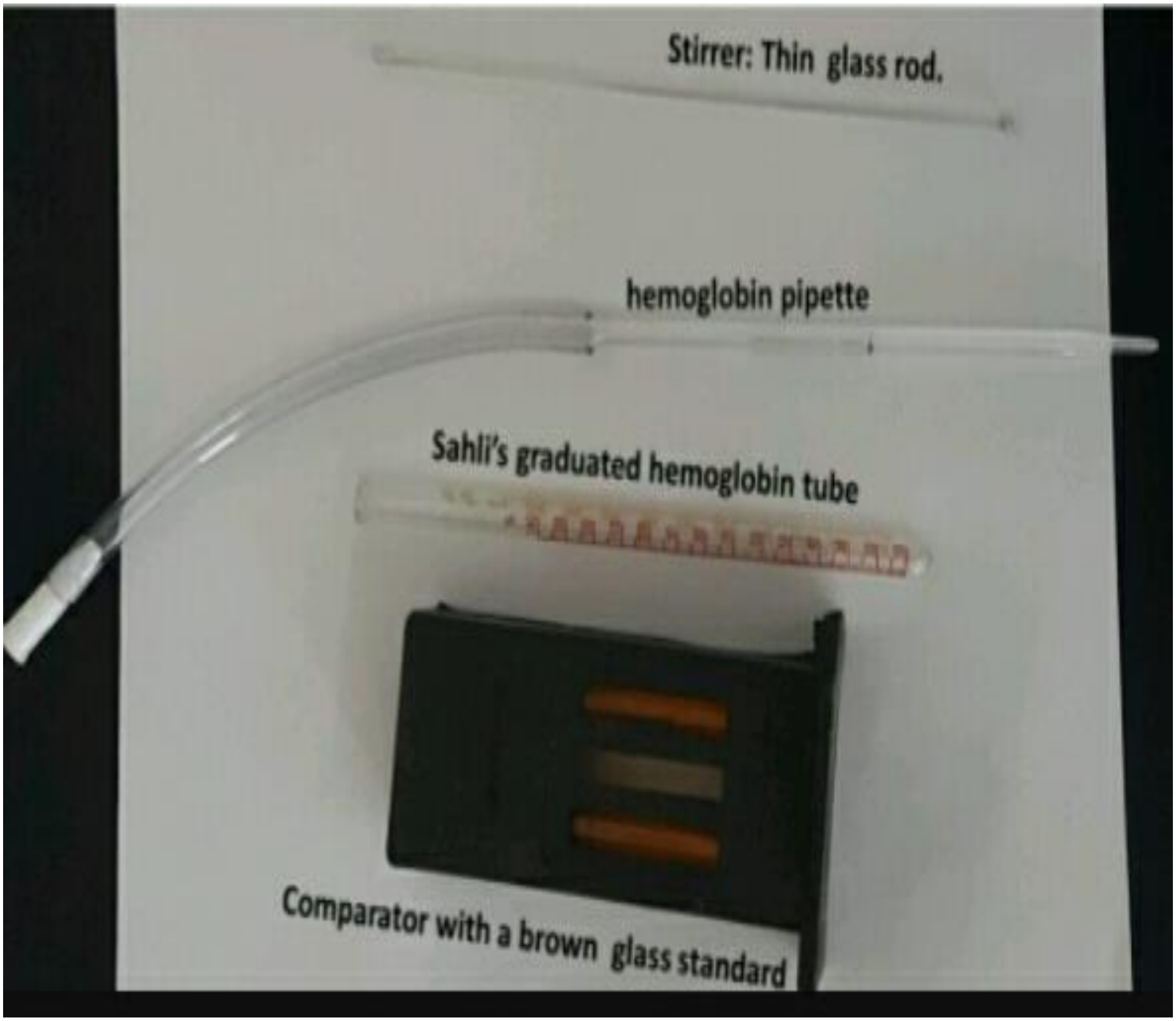
2


Stirrer: Thin glass rod.
hemoglobin pipette
Sahli's graduated hemoglobin tube
Comparator with a brown glass standard