



Growth and physiological variables of nesting and laying domestic hens

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Abstract

The study was aimed at evaluating the growth and physiological parameters of nesting and laying domestic hens. Fifteen broody and laying hens (56 wk of age, 36 wk in lay) were used for the study which lasted for 4 weeks. The broody birds were weighed and then housed individually in floor pens equipped with nest boxes and hard boiled eggs to encourage incubation behaviour while the laying hens were housed in 3 replicate pens (5 birds/pen). Parameters measured were initial (IBW) and final (FBW) body weight, daily (DBWG) and cumulative (CBWG) body weight gain, weekly feed intake (WFI), average daily feed intake (ADFI) and haematological indices: packed cell volume (PCV), red blood cell (RBC) count, haemoglobin concentration (Hb), white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and differential white blood cell count. Others were ambient temperature (AT), body temperature (BT), and respiratory rate (RR) recorded at three time periods (TP): 07:00-08:00h, 13:00-14:00h and 18:00-19:00h for 3 days/wk for 3 wk. FBW, CBWG, ADFI and RR, some erythrocytic indices but not DBWG, AT, BT, leukocytic indices, and H/L ratio were affected by broody or laying status. Time period did not influence BT, and RR of the hens. FBW, DBWG, PCV, Hb, and RBC varied significantly ($P<0.05$) in the nesting group. It was concluded that nesting is associated with significant physiological stress and altered haematological and growth parameters and that nesting and laying hens have equivalent physiological stress profile.

Keywords: Body temperature; haematological indices; H/L ratio; respiratory rate; stress

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Introduction

Broodiness or nesting behaviour is characterised by anorexia, stoppage of laying, and vigorous defence of the nest (Romanov, 2001; Cunningham and Klein, 2007). It is observed in most breeds of domestic chicken with the exception of White Leghorn (Romanove et al., 2002). Commercial breeds which are products of intensive selection do not go broody as frequently as unimproved breeds (Romanove, 2001).

Broodiness evolved for successful reproduction in the free range poultry. On account of its fundamental role in avian reproduction, broodiness or incubation behaviour has been of great interest to poultry scientists (Romanove, 2001). Several studies (El-Halawani et al., 2000; Romanove et al., 2002; Reddy et al., 2006;

Kagya-Agyemang et al., 2012) have investigated the genetic, physiological and hormonal basis of incubation behaviour, as well as its effect on productive traits. Elevated plasma prolactin maintained by tactile stimulation from eggs or chicks and the brood patch (a bare area of skin in contact with incubated eggs) is required to maintain broodiness (Vleck et al., 2000; Romanove, 2001).

Selection for increased egg production in the exotic breeds of chicken led to decreases in broodiness (Romanove et al., 2002). Thus Broodiness could be partly responsible for the poor laying performances of unimproved indigenous chicken strains in Nigeria. Broodiness causes frequent egg pauses, poor laying performance (Reddy et al., 2006; Eltayeb et al., 2010), and weight loss (wasting) (Cunningham and Klein,

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2007; Biobakun and Adeleye, 2010). Genetic selection, moving hens to unfamiliar pens, frequent collection of eggs, and provision of uniform lighting in housed chickens help to reduce the incidence of broodiness. Hormonal and chemical mechanisms to control broodiness include immunization with synthetic chicken vasoactive intestinal peptide (cVIP) antibodies, cyproheptadin (a serotonin or 5-HT receptor antagonist) or chlorpromazine (a dopamine receptor antagonist) (Kagya-Agyemang et al., 2012), and feeding of bromocriptine (a prolactin suppressant) (Reddy et al., 2006).

Broodiness and incubation of eggs could be associated with much stress. Jakubas et al. (2008) stated that egg incubation is an important component of reproductive costs in chickens. Energetic constraints imposed by broodiness constitute stress factors which may be reflected in altered physiological parameters such as metabolic rate and blood profile. Variations in blood parameters namely heterophils (H), lymphocytes (L), and H/L ratio have been used as reliable indices of stress in avians (Ruiz et al., 2002; Davis et al., 2008; Jakubas et al., 2008; Gladbach et al., 2010). The H/L ratio is not affected by stress associated with animal handling and blood sampling and it has been shown to be more stable than plasma corticosteroid level as a measure of stress in avians (Jakubas et al., 2008). We predict that the metabolic rate (measured by body temperature and respiratory rate), growth performance and haematological profile of the nesting hen reflect the stress associated with nesting behaviour. We also predict that the stress profile of the nesting hen is higher than that of the laying hen given the freedom to acquire feed and absence of nest defence in the later. Accordingly, the present study was carried out to (1) evaluate the growth parameters (BW, FI, and BWG) of broody and laying domestic hens (2) evaluate the effect of nesting and laying status on body temperature (BT), respiratory rate (RR), and haematological indices of the birds and (3) compare the stress profile (H/L ratio) of nesting and laying hens as measures of reproductive costs in the Nigerian indigenous chickens.

Materials and Methods

Location and duration of the study

The experiment was carried out at the local chicken unit of the poultry farm of the Department of Animal Science, University of Nigeria, Nsukka latitude 5° 24' North and longitude 7° 24' East with mean daily ambient temperature and relative humidity range of 27.7°C to 32.7°C and 64.5 to 70.0%, respectively (Okenyi et al., 2013). The study lasted for 4 weeks.

Experimental birds and management

Broody and laying (non-broody) indigenous chickens (15/group) were selected from a population of local chickens in the Department of Animal Science, University

of Nigeria, Nsukka. The birds were 56 wk of age and 36 wk in lay at the commencement of the study, respectively. The birds were weighed to obtain their initial body weight. The broody birds were then housed individually in floor pens equipped with nest boxes and hard boiled eggs to encourage incubation behaviour (Ramanove et al., 2002) while the laying hens were housed in 3 replicate pens (5 birds/pen). The broody hens were given free access to a layer ration having 16.5% crude protein and 2650 kcal/kg of metabolizable energy while the laying hens were fed 125g/bird/day of the same layer feed as determined previously by Ogbu (2012). Water was provided at all times to the two groups. Routine health management practices which included prophylactic antibiotic and vitamin medications, deworming and delousing were carried out during the experiment to ensure optimal health of the experimental birds. Each broody hen was allowed to sit on the hard boiled eggs for 3 wk starting from the day the bird incubated the eggs for most of the day. A hen that abandoned the eggs (broody group) or became broody (laying group) during the study was removed from the experiment.

Data collection

Data collected were initial body weight (IBW), weekly body weight (WBW), final body weight (FBW), daily body weight gain (DBWG), average daily feed intake (ADFI), ambient temperature (AT) of pen (laying group) and nest boxes (broody group), body temperature (BT), respiratory rate (RR) and haematological parameters measured or determined (packed cell volume, PCV; red blood cell count, RBC; haemoglobin concentration, Hb; white blood cell count, WBC; mean corpuscular volume, MCV; mean corpuscular haemoglobin, MCH, mean corpuscular haemoglobin concentration, MCHC, and differential WBC count). AT, BT and RR were taken three times daily within time periods 07:00–08:00 h, 13:00–14:00 h and 18:00–19:00 h, respectively for 3 d per wk for 3 wk. AT was measured by means of a thermometer hung within each pen (laying group) and in the nest boxes (broody group). BT was obtained as cloacal temperature using a digital thermometer held against the mucosa of the cloaca until the reading stabilized. RR was obtained by counting the raising of the abdomen/min while the hen was at rest (Pampori and Iqbal, 2007; Nascimento et al., 2012). Blood sample was drawn from each bird into EDTA bottles and used for the determination of haematological parameters. Sampling was done at onset of persistent incubation behaviour (wk 0), and at wk 1, 2 and 3 (end of incubation) as well as at wk 4 (1 wk after removal of eggs from the broody hens).

Statistical analyses

Data collected were submitted to Repeated Measures Analysis of variance. Time period and week were treated as within subjects factors in separate analysis while

physiological status was treated as a between subjects factor. In the analysis for effect of time period, week was included as a covariate. The statistical model was:

$$X_{ijk} = \mu + S_i + T_j + W_k + \epsilon_{ijk}$$

Where, X_{ijk} =an observation, μ =overall mean, S_i =effect of status, T_j =effect of time period, W_k =effect of week, and ϵ_{ijk} =Random error. Data for nesting (broody) and laying groups were analyzed separately. Significantly different means at 95% probability were separated using the Duncan New Multiple Range Test (Duncan, 1955). Only data from birds that remained nesting on eggs (n=13) or did not nest on eggs (n=12) till the end of the experiment were included in the analysis. Comparison between broody and laying hens was done using independent samples t-test.

Results and Discussion

During the period of incubation, 2 birds from the broody group abandoned their eggs while 3 birds showed signs of incubation behaviour. These birds were removed from the experiment and data on them were not included in the analysis. The environmental, physiological, and performance variables of nesting and laying domestic hens are presented in Fig. 1. There were significant ($P < 0.05$) differences in RR, FBW, and ADFI of the experimental birds but not in ambient temperature (AT), body temperature (BT) or daily body weight gain (DBWG).

Mean AT of the nest boxes and laying pens were 30.22 ± 0.24 and $29.67 \pm 0.26^\circ\text{C}$, respectively ($P > 0.05$) indicating similar thermal environment for the two experimental groups. The BT for nesting and laying hens were 41.64 ± 0.08 and $41.72 \pm 0.07^\circ\text{C}$, respectively ($P > 0.05$). There is dearth of information on the body temperature and respiratory rates of nesting (broody) domestic hens in literature but our values for body temperature agree with those reported by Ilori et al. (2012) for non broody Nigerian indigenous hens (41.27 ± 0.02 , 41.19 ± 0.03 , and $40.10 \pm 0.01^\circ\text{C}$ for normal feathered, naked neck, and frizzle genotypes, respectively) and by Aengwanich (2008) for non broody Thai indigenous chickens (40.88 – 41.49°C for cocks, and 40.75 – 41.46°C for hens) reared under normal ambient temperatures indicating that nesting or laying did not significantly influence body temperature. This also shows that the range of AT (29.67 to 30.22°C) observed in the present study were within the thermoneutral zones of the birds. Thus both nesting and laying hens did not need to significantly alter physiology (e.g., metabolic rate) to maintain normothermia. Homeotherms generally alter physiology to maintain homeostasis when AT significantly deviates from their range of thermal comfort (Ogbu et al., 2013). Kunz and Orrell (2004) also reported

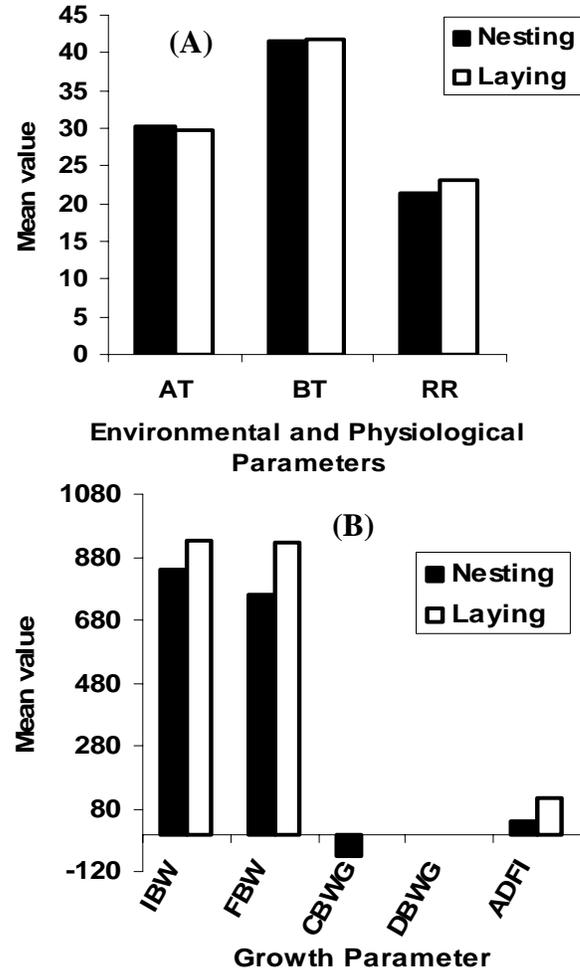


Fig. 1: Environmental and physiological (a) and growth parameters (b) of nesting and laying domestic hens. AT: ambient temperature ($^\circ\text{C}$); BT: body (cloacal) temperature ($^\circ\text{C}$); RR: respiratory rate (count/min); IBW: initial body weight (g); FBW: final body weight (g); CBWG: cumulative body weight gain (g); DBWG: daily body weight gain (g); ADFI: average daily feed intake (g).

that when environmental temperatures are below the thermoneutral zone of nesting hens, they raise their metabolic rates to provide enough heat to maintain egg temperature. The similarity in the BT of the experimental groups reflects equivalent thermoregulatory capacity in both nesting and laying hens. It also suggests that both nesting and laying hens engage in trade-off of energy resources – one towards egg production, the other towards chick production-without significant alteration in metabolic rate. The nesting hen actually raises her metabolic rate periodically (especially after an off-bout) to generate enough body heat to restore egg temperature (Brummermann and Reinertsen, 1992). Nesting hens had significantly ($P < 0.05$) lower RR (21.48 ± 0.42 counts/min) compared to laying hens (23.00 ± 0.45 counts/min) which

could be attributed to reduced physical activity, reduced metabolic rate, or lesser thermal stress compared to the layer group. The RR values, however, correspond more to the lower limit of range of values for RR reported for healthy indigenous chickens under ambient temperatures (Pampori and Iqbal, 2007; Ilori et al., 2012). This may be ascribed to differences in age, physiological status and environmental differences all of which influence physiological values (Davis et al., 2008). DBWG did not differ significantly ($P < 0.05$) according to physiological status even though nesting hens lost about 2.35 ± 0.67 g/day compared to the 0.61 ± 1.39 g/day for laying hens. The lack of statistical significance could be due to the small number of birds involved as well as the large standard error of means. Nesting hens, however, had significantly ($P < 0.05$) higher cumulative body weight loss (negative CBWG) compared to laying hens (-72.43 g or 8.68% vs -1.43 g or 0.15%). Nesting hens have been reported to lose weight due to severe wasting of muscles (Cunningham and Klein, 2007; Biobakun and Adeleye, 2010) probably due to physiological anorexia. Berry (2003) reported that the jungle fowl hen losses about 20% of its body weight during incubation. The losses in BW is a trade-off between maintenance of body condition and reproductive success (parental investments to increase fitness) (Kunz and Orrell, 2004). Nesting hens consumed an average of 39.28 ± 1.36 g of feed per day compared to 110.97 ± 1.30 g/day for layers ($P < 0.05$) which agrees with the report by Biobakun and Adeleye (2010) that nesting hens feed sparingly. Eltayeb et al. (2010) reported a reduction in feed intake of between 47 and 50% during broodiness compared to the value during egg production. The highly reduced feed intake of nesting hens resulted from reduced appetite (anorexia) caused by hormonal effects on the satiety nuclei of the hypothalamus (Cunningham and Klein, 2007; Biobakun and Adeleye, 2010). Berry (2003) also reported that incubation of eggs in the jungle fowl is accompanied by spontaneous anorexia while Cooper and Voss (2013) reported that broody hens allocated very short time to feeding during off-bouts to minimize egg cooling. The restricted feed intake constrains energy acquisition which results in the mobilization of body reserves (fat deposits and muscle mass) to maintain body metabolism at a rate necessary to generate enough body heat for incubation and thermoregulation (Kunz and Orrell, 2004). The significant ($P < 0.05$) differences between nesting and laying hens in growth parameters (FBW, CBWG, and ADFI) indicate that cost of incubation could be a significant component of total cost of reproduction in the nesting domestic fowl (Kunz and Orrell, 2004).

Physiological status significantly ($P < 0.05$) affected the erythrocytic indices (PCV, HbC, RBC, and MCH) (Fig. 2a) but not the leukocytic indices and H/L ratio (Fig. 2b). The laying group had higher values of PCV, HbC, and RBC compared to the nesting hens (30.13 ± 1.36 vs

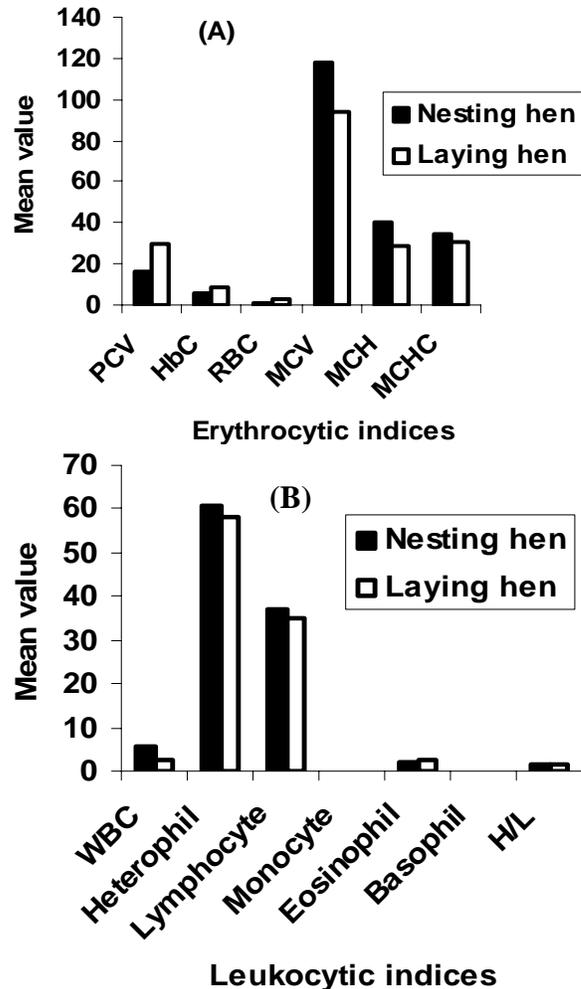


Fig. 2: Erythrocytic (a) and leukocytic (b) indices of nesting and laying domestic hens. PCV: packed cell volume (%); haemoglobin concentration (g/dl); RBC: red blood cell ($\times 10^6$); MCV: mean corpuscular volume (fl); MCH: mean corpuscular haemoglobin (pg); MCHC: mean corpuscular haemoglobin concentration (g/dl); WBC: white blood cell ($\times 10^3$); H/L: heterophil: lymphocyte ratio.

$16.13 \pm 1.20\%$, 9.08 ± 0.64 vs 5.52 ± 0.15 g/dl and 3.28 ± 0.30 vs $0.89 \pm 0.18 \times 10^6$ /ul, respectively) while nesting hens had higher values for MCH (40.46 ± 1.95 vs 28.63 ± 4.02 pg). The lower PCV, HbC, and RBC values in the nesting hens could be as a result of nutrient deficiency (due to the lower feed intake) in this group which could lead to reduced erythropoiesis. In addition, the lower physical activity due to incubation and the need to conserve energy could mean lesser oxygen demand by body cells and concomitant reduction in circulating RBCs and blood HbC. Energy savings are necessary for successful incubation and thermoregulation (Kunz and Orrell, 2004; Jakubas et al., 2008). The higher MCH in broody hens could be compensatory due to the reduced circulating

RBC. The non significant differences in leukocytic indices show that circulating WBCs (leukocytes) in the hens at the time of blood assay were similar. We found the predominant circulating leukocytes in nesting and laying hens to be heterophils (60.75 ± 0.85 , and $58.00 \pm 3.56\%$, respectively) followed by lymphocytes (37.25 ± 0.85 , and $35.00 \pm 2.65\%$, respectively) and then eosinophils (2.25 ± 0.25 , and $2.25 \pm 1.19\%$, respectively) which agree with the reports of Jakubas et al. (2008) and Gladbach et al. (2010). The H/L ratio for the groups was 1.63 ± 0.06 and 1.66 ± 0.05 for nesting and laying hens, respectively. Comparison of the leukocytic indices and H/L ratio with values for birds determined to be under low and optimal stress (Pampori and Iqbal, 2007; Melesse, 2011; Ogbu et al., 2013) indicate that both the nesting and layer chickens in the present study were under considerable stress. It has been established that stress causes a rise in heterophils (heterophilia) and reductions in lymphocytes (lymphopenia) and eosinophils (eosinopenia) in birds (Eeva et al., 2005; Davis et al., 2008; Melesse, 2011; Azis, 2012). Lymphocytes and neutrophils (or heterophils in avians) (more than other blood cells) undergo redistribution in response to increased blood glucocorticoid levels during stress (Dhabhar, 2002; Davis et al., 2008). While lymphocytes move out of the blood stream into lymphoid organs, bone marrow and skin to be sequestered, neutrophils or heterophils move into the blood stream (Dhabhar, 2002). The H/L ratios were closer to values reported for birds under high stress (range, 0.8–2.55) (Gross and Siegel, 1983; EL-Lethey et al. 2003; Campos et al., 2006). The equivalent H/L ratio for nesting and laying hens was surprising. We had expected nesting hens to manifest evidence of higher stress than layers. It could be that the hormonal and physiological mechanisms responsible for initiation and maintenance of incubation behaviour provide for homeostatic buffering (physiological blunting) of the stress effects of such physiological processes (Berry, 2003; Webster, 2003).

The effect of time period on environmental and physiological variables of the experimental birds are presented in Fig. 3.

Period of day significantly ($P < 0.05$) influenced AT in both nest boxes and laying pens showing that daily fluctuations in weather condition affected the micro environment of the experimental birds. Ambient temperature was highest in time period 13:00–14:00h (afternoon) ($31.72 \pm 0.12^\circ\text{C}$ for nest boxes and $31.67 \pm 0.12^\circ\text{C}$ for laying pens) followed by 18:00–19:00h (dusk) ($30.44 \pm 0.13^\circ\text{C}$ for nest boxes and $29.56 \pm 0.13^\circ\text{C}$ for laying pens) but lowest within 07:00–08:00h (morning) ($29.61 \pm 0.26^\circ\text{C}$ for nest boxes and $28.89 \pm 0.26^\circ\text{C}$ for laying pens) probably on account of differences in insolation and air circulation at these periods of the day. Time period 13:00–14:00h (afternoon) corresponds to the period of highest insolation in the experimental area.

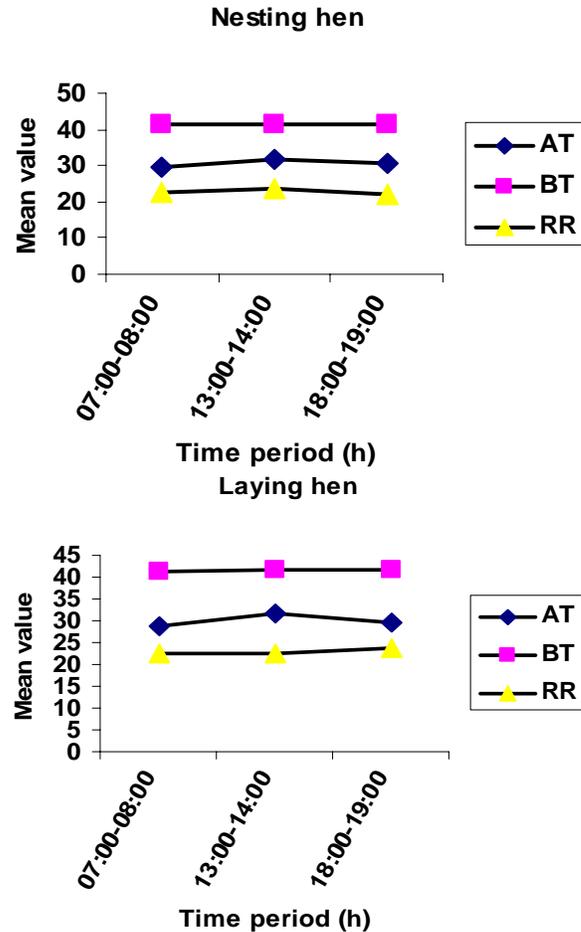


Fig. 3: Time period effects on environmental and physiological variables of nesting and laying hens. AT: ambient temperature ($^\circ\text{C}$); BT: body (cloacal) temperature ($^\circ\text{C}$); RR: respiratory rate (count/min).

Time period did not significantly ($P > 0.05$) affect BT and RR in both nesting and laying hens showing that birds in each group maintained normal metabolic rate within the range of ATs observed. These parameters though not significantly different with time period increased during the period of high AT (13:00–14:00h) showing that AT influences physiological parameters in animals (Altan et al., 2000). The insignificant differences in BT and RR (under varying AT) reflect the ability of the body to buffer body temperature and maintain metabolic rate within normal range in order to maintain normothermy and homeostasis and that the AT values were within the thermoneutral zone of the birds. The range of values reported for BT in the three time periods agree substantially with those of Ilori et al. (2012) and Isidahomen et al. (2012) who reported a range of 40.10 ± 0.01 to $41.27 \pm 0.02^\circ\text{C}$ and 40.09 ± 0.21 to $41.68 \pm 0.03^\circ\text{C}$, respectively in three genotypes (normal feathered, naked-neck and frizzled-feathered) of the Nigerian indigenous chicken. The values obtained for RR

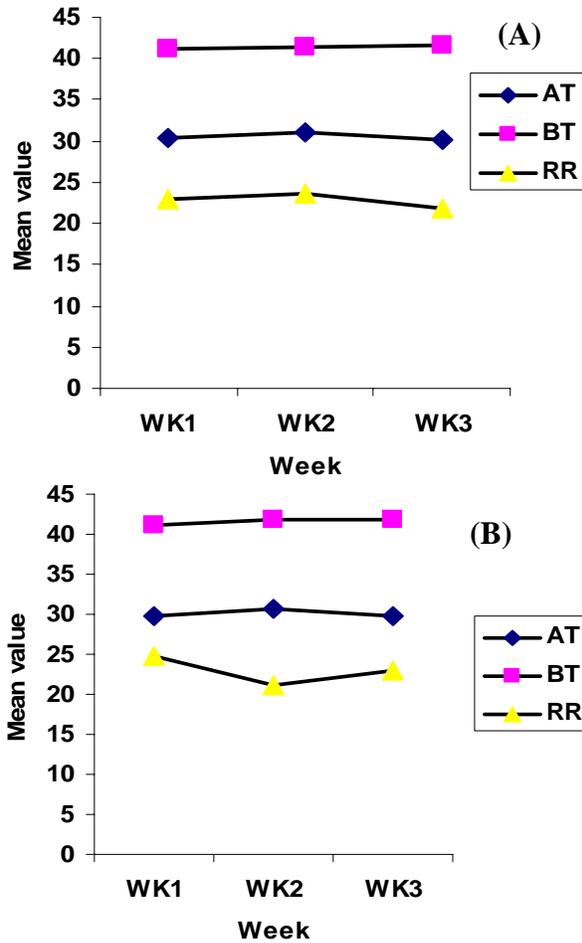


Fig. 4: Weekly trend in environmental and physiological variables of nesting (a) and laying (b) domestic hens. AT: ambient temperature (°C); BT: body temperature (°C); RR: respiratory rate (counts/min).

correspond to the values of 22.00 ± 1.49 breath/min reported for frizzled feathered native chicken by Isidahomen et al. (2012) but quite lower than 64.80 ± 2.60 breath/min reported for naked-neck chickens by the same authors. The values are also lower than those reported by Ilori et al. (2012) and Pampori and Iqbal (2007) for native chickens of Kashmir. These wide differences could be due to phenotypic differences in body size, age, environment, and physiological status.

The weekly values for environmental and physiological variables, and growth parameters of the experimental birds are presented in Fig. 4, and 5, respectively. AT and BT did not differ significantly ($P < 0.05$) with wk of experiment in nesting (Fig. 4a) and laying (Fig. 4b) groups. Isidahomen et al. (2012) reported stable rectal temperature of three indigenous chicken genotypes over a 24 wk experimental period. The significantly ($P < 0.05$) higher RR of 23.67 ± 0.70 counts/min for nesting hens at wk2 compared to wk3 may not be

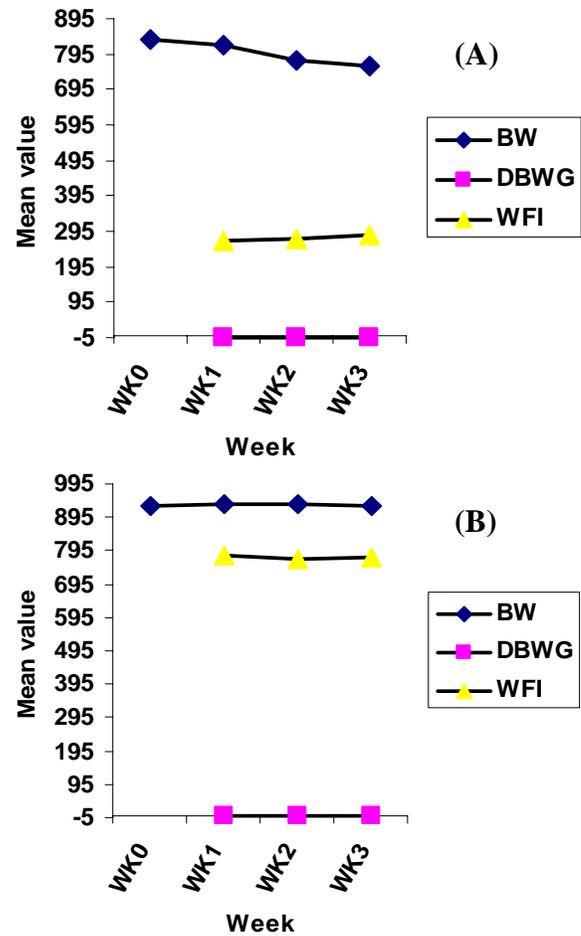


Fig. 5: Weekly trend of growth parameters of nesting (a) and laying (b) domestic hens. BW: body weight (g); DBWG: daily body weight gain (g); WFI: weekly feed intake (g).

biologically significant or could be due to higher physical activities (e.g., more frequent off-bouts) needed to replenish energy resources. We observed decreased off-bouts as incubation progressed. A contrary result was obtained for the laying hens in which RR was least ($P < 0.05$) at wk2 although we could not adduce any reason to this. Generally, fluctuations in RR over weeks would be expected under uncontrolled environmental variables and temporary changes in the rate of body metabolic activities. Growth parameters (BW, BWG and WFI) did not change significantly ($P < 0.05$) across the experimental period in the laying group (Fig. 5b) unlike in the nesting group in which BW and BWG varied significantly ($P < 0.05$) across the experimental period (Fig. 5a). BW was least ($P < 0.05$) at wk3 of incubation in the nesting group (762.14 ± 27.03 g) due to cumulative weight loss. The highest value for weight loss (negative body weight gain) (-6.12 ± 0.86 g) occurred at wk2 of incubation which probably represents the period of greatest mobilization of

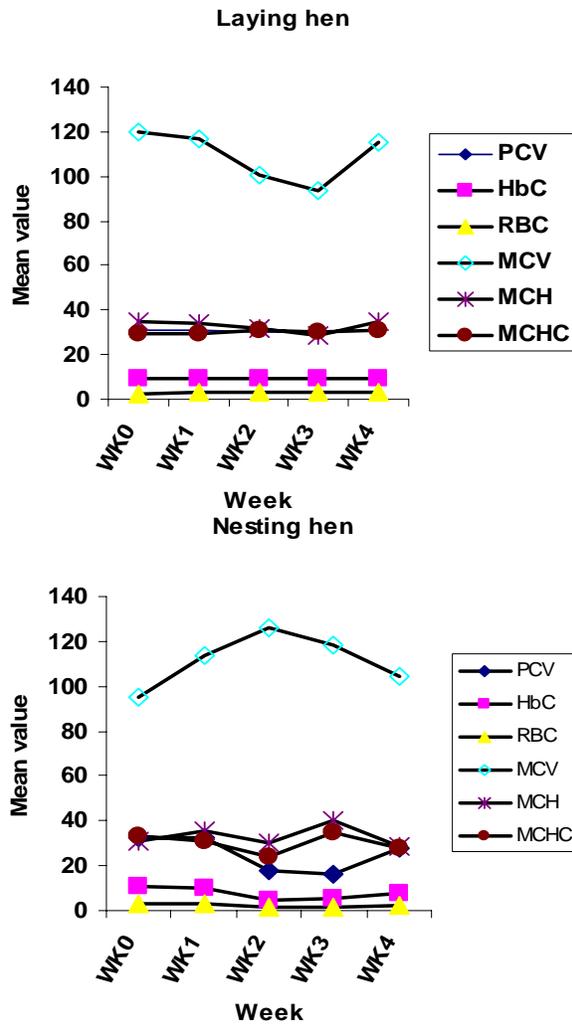


Fig. 6: Weekly trend in erythrocytic indices of nesting (a), and laying (b) domestic hens. PCV: packed cell volume (%); haemoglobin concentration (g/dl); RBC (x10⁶/μl): red blood cell; MCV (fl): mean corpuscular volume; MCH: mean corpuscular haemoglobin (pg); MCHC: mean corpuscular haemoglobin concentration (g/dl)

body reserves in this group. The non-significant ($P < 0.05$) variation in WFI in both nesting and laying groups indicate that the birds used for the study have attained optimal growth and feed requirement so that daily (or weekly) requirements remained virtually constant. For the nesting hens, these further shows that the hens fed more during each feeding bout as incubation advanced (Cooper and Voss, 2013).

The weekly values for the erythrocytic and leukocytic profiles of the experimental groups are presented in Fig 6, and 7, respectively. Significant ($P < 0.05$) differences were observed in some erythrocytic (PCV, HbC, RBC, and MCHC), and leukocytic (eosinophils) indices of nesting hens but not in those of laying hens. In nesting hens,

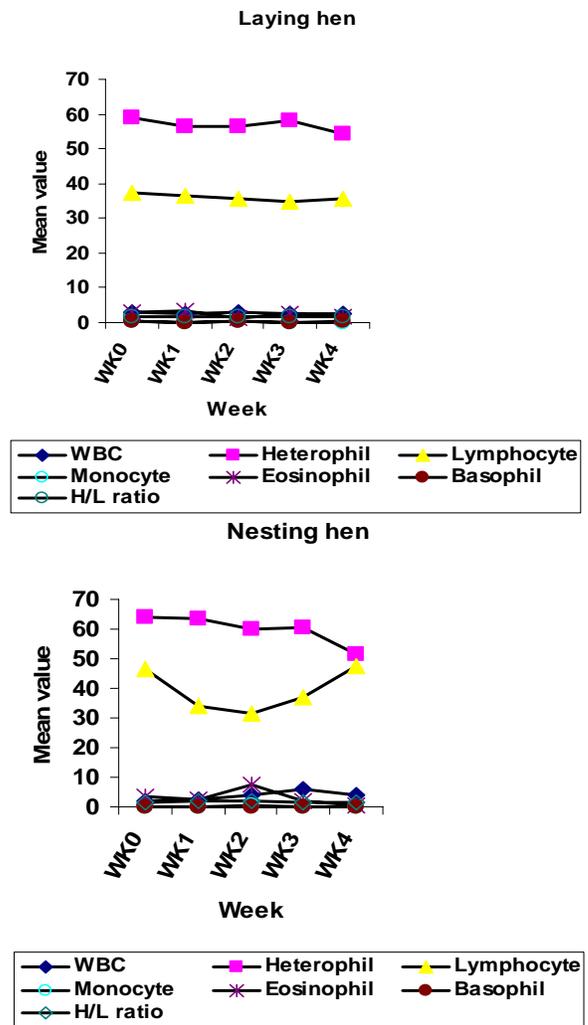


Fig. 7: Weekly trend in leukocytic indices of nesting (a), and laying (b) domestic hens. WBC: white blood cell; H/L ratio: heterophil:lymphocyte ratio.

PCV, HbC, and RBC decreased over the incubation period with the least values at wk2, and 3 for PCV ($18.00 \pm 1.07\%$, and $16.13 \pm 1.28\%$, respectively), HbC (4.38 ± 0.77 g/dl, and 5.52 ± 0.47 g/dl, respectively), and RBC ($1.46 \pm 0.23 \times 10^6$ /ul, and $1.37 \pm 0.21 \times 10^6$ /ul, respectively), and wk 2 for MCHC (24.04 ± 2.54 g/dl) while eosinophils were highest at wk 2 ($7.75 \pm 0.95\%$). Thus, PCV, HbC, and RBC decreased to between 55.59 and 49.81, 52.08 and 41.32, and 42.31 and 39.71% of their initial (wk0) values, respectively while MCHC, and eosinophils presented no definite trend. These parameters however increased to between 71.97 and 85.33% of their wk0 values one week after removal of incubated eggs (i.e., wk4) probably on account of increased feeding, and recovery of good body condition. The fluctuating pattern of MCHC, eosinophils, and some leukocytes agree with the report of Owen and Sogge (2002) that fluctuations in

types of leucocytes indicate stress on the system by a non-etiological process such as malnutrition or significant water loss from the body.

Conclusion

There were no significant alterations of basal metabolic rate in the nesting hen as reflected in the BT and RR of the hens. Again, we did not observe significant differences in stress profile between nesting and laying hens as both groups revealed equivalent H/L ratio. Nesting was however associated with significant reduction in growth parameters (BW, BWG, and FI). Furthermore, both nesting and laying involved significant physiological stress reflected in altered erythrocytic, and leukocytic indices as well as raised H/L ratio.

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