Physiological Adaptation of Sasso Laying Hens to the Hot-Dry Tropical Conditions

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Summary

The study aimed at evaluating the adaptive physiological capacity of Sasso laying hens in a hot-dry environment. Fifty four randomly selected 30-week old Sasso layers were each kept on deep litter and in battery cages. General Linear Model was used to determine the fixed housing system and age (42 and 52 weeks old, respectively) effects including their interaction with thermo-physiological traits [rectal temperature (RT), respiratory rate (RR), pulse rate (PR) and heat stress index (HSI)] while Exhaustive CHAID algorithm was used to predict HSI. Haematological and serum biochemical indices considered were subjected to T- test at 52 weeks of age. The mean environmental Temperature-Humidity index (79.92) indicated that the birds experienced thermal discomfort. Univariate analysis revealed that birds kept in battery cages appeared to be more stressed with higher mean values ($P<0.05$) for PR (144.32±0.55 versus 142.54±0.52), RR (31.91±0.42 versus 29.94±0.41) and HSI (1.76±0.03 versus 1.85±0.03). However, age effect ($P<0.05$) was only observed in RT and PR and varied from one housing system to the other. There was housing system and age interaction effect on RT, RR and HSI. Exhaustive CHAID regression revealed that HS1 could better be predicted by RR and age. While haematological parameters were not influenced ($P>0.05$) at 52 weeks of age, serum cholesterol and glucose were higher ($P<0.05$) in birds reared on deep litter. The present findings could facilitate the formulation of appropriate management strategies towards combating heat stress and aid the conservation and genetic improvement of Sasso chicken under hot-dry conditions.

Key words

thermoregulation, Sasso, tropics, blood parameter, regression

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Introduction

Periods of high environmental temperatures and high relative humidity are common in the hot-dry season in the tropics, subtropics and mild climates (Šleger and Neuberger, 2006; Mader et al., 2010). Exchange of heat could directly be accessed from some physiological measurements such as rectal, cloacal and skin temperatures, respiratory rate, panting, and heat production (Renaudeau et al., 2012). Birds are more susceptible to sudden, large changes in temperature (Safdar and Maghami, 2014). Of a major concern to both backyard and commercial poultry farmers is the deleterious effect of heat stress on the welfare and performance of birds (Rath et al., 2015; Sugiharto et al., 2017) that may result in economic losses in the farms. When birds' body temperatures are in their thermoneutral zone, the energy can be directed to growth, immune system development and reproduction. However, during the period of heat stress, birds strive to adjust by making several physiological changes to maintain body temperature, including electrolyte depletion and reduced immune response (Etches et al., 2008). According to Mashaly et al. (2004), the humoral immunity of laying hens is generally depressed in hot conditions, including decreases in total white blood cell count and antibody production. Changes in thermo-physiological traits (El-Tarabany, 2016), haematological and biochemical indices (Ladokun et al., 2008; Panigrahy et al., 2017) can be used to evaluate the physiological or immune status, and hence, the degree of adaptation of birds to hot-dry conditions (Chaiyabutr, 2004).

The understanding of the animal responses to thermal challenge is paramount to successful implementation of breeding strategies to increase production and productivity of birds under warm climate (Renaudeau et al., 2012). In recent times, interest in the utilization and conservation of tropically adapted birds has been renewed. Sasso chickens were developed in France and are known for heat tolerance and ranging ability. Existing preliminary records (Yakubu and Ari, 2017) showed better performance of this newly introduced strain when compared with an indigenous chicken counterpart in a sub-humid environment in the tropics. However, there appears to be no documented information on the heat tolerance traits and blood parameters of Sasso birds in the literature. Knowledge of these will complement subsequent characterization efforts and aid effective management of the birds.

The present study, therefore, aimed at evaluating the thermo-physiological traits, haematological and serum biochemical indices of Sasso laying birds in a hot-dry tropical environment.

Materials and methods

International Council for Laboratory Animal Science and NC3Rs ARRIVE (Animals in Research: Reporting *In Vivo* **E**xperiments) guidelines on research ethics were strictly followed.

Experimental site

This experiment was carried out at the Livestock Section of the Teaching and Research Farm of the Faculty of Agriculture, Nasarawa State University, Keffi, Shabu-Lafia Campus. It is located within the guinea savannah zone of North Central Nigeria and lies on latitude 08° 35N and longitude 08° 33E, respectively.

Experimental birds and their management

A total of one hundred and eight birds (108) 30 weeks old Sasso hens were randomly selected from a larger stock kept at the Livestock Farm. Fifty four birds each were kept on deep litter and in battery cages with open ventilation. The birds in each system of management were randomly divided into three replicates, each containing eighteen birds. Each bird in each system was tagged with an identification number. The birds were fed commercial layer mash (Vital Feed) from week 30 to week 52 of rearing and supplied clean water *ad libitum*. There was strict adherence to routine vaccination and medication. Newcastle Disease Virus (NDV) booster vaccine was orally administered to the birds when they were 30 weeks of age. Antibiotics were given at 36-week old. Oral administration of vitamins was done at days 233, 252 and 267. Other management practices were carried out as described by Yakubu et al. (2010).

Meteorological data collection

Air temperature, dew point, relative humidity, rainfall, solar radiation, pressure and wind speed records were taken from the 30^{th} to 52^{nd} week of the experiment. The ambient temperature and the relative humidity readings were used to determine the Temperature-Humidity index in accordance with the following formula (Moraes et al., 2008):

$$0.8\ T_{db} + [RH\ (T_{db}-14.3)/100] + 46.3$$

where:

T_{db}= air dry-bulb temperature (°C); RH= relative humidity of air (%).

Thermo-physiological data collection

At 42 weeks of age, Rectal temperature (RT), Respiratory rate (RR), and Pulse rate (PR) were taken in the morning (between the hours of 7:00 am and 9:00 am) and in the afternoon (1:00 pm and 3:00 pm) consecutively for three days. The same measurements were repeated when the birds were 52 weeks old. All surviving birds in each housing system and age were measured as indicated in Table 1 below.

Table 1. Number of birds in each housing system at a particular age

Age (weeks)	System	
	Deep litter	Battery Cage
42	44	40
52	43	40

RT, RR and PR were determined following the procedures outlined in an earlier study (Ilori et al., 2011). Heat stress index (HSI) was calculated using the following formula described by Adedeji et al. (2015):

$$HSI = AR/AP \times NP/NR$$

where:

AR = Actual respiratory rate; AP = Actual pulse rate; NP = Normal pulse rate and NR = Normal respiratory rate.

Haematological and serum biochemical indices determination

At week 52 of age, blood samples (about 2 ml) were collected from the wing vein of four randomly selected birds per management system with a 21G needle connected to a 5ml syringe. Blood samples were placed in two tubes, one containing a coagulant [ethylenediaminetetraacetic acid (EDTA)] to determine haematological parameters and the other without anticoagulant and left to clot for serum biochemical analysis. Packed Cell volume (PCV), Haemoglobin (Hb) concentration, red blood cell (RBC) and white blood cell (WBC) counts were determined according to the methods of Dacie and Lewis (1991). Mean cell volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) were calculated as described by Jain (1986). Serum total proteins and albumin were determined using the procedures outlined by Tietz (1995). Globulin was calculated by subtracting serum albumin from serum total protein for each bird. Serum cholesterol was measured following the method of Allain et al. (1974) while glucose determination was in accordance with the procedure of Tietz (1995).

Statistical analyses

Three models were used to analyse the data obtained in this study. The first involved the use of General Linear Model (GLM) procedure in a completely randomized design to test the fixed effects of housing system and age including housing system and age interaction on rectal temperature, respiratory rate, pulse rate and heat stress index. Least Significant Difference (LSD) procedure was used to separate the means at 95% confidence interval. The linear additive model employed was:

$$Y_{ijk} = \mu + H_i + A_j + (HA)_{ijk} + e_{ijk}$$

where:

Y_{ijk} = individual observation; μ = general mean; H_i = fixed effect of i^{th} housing system (i = deep litter, battery cage); A_j = fixed effect of j^{th} age (j = 42 weeks, 52 weeks); $(HA)_{ijk}$ = housing system and age interaction effect; e_{ijk} = random error associated with each error

The second model involved the use of Exhaustive chi square automatic interaction detector (CHAID) that examines all possible splits for each predictor. Here, the response variable (dependent variable) was heat stress index, while the predictors (explanatory or independent variables) were rectal temperature, respiratory rate, pulse rate, housing system and age. The Exhaustive CHAID was subjected to Bonferroni adjustment of significance values. The outcome was validated using 10-fold cross-validation. In the third model, T-test analysis was used to compare the thermo-physiological traits (rectal temperature, respiratory rate, pulse rate and heat stress index), haematological parameters (RBCs, WBCs, Hb and PCV) and serum biochemical characteristics (total protein, albumin, globulin, cholesterol and glucose) of birds kept on deep litter and in battery cages at 52 weeks of age. All analyses were carried out using SPSS (2015) statistical package.

Results

The mean values (±S.E.) for the meteorological parameters generated digitally from March-June, 2017 are shown in Table 2. As the air temperature increased, the relative humidity decreased, indicating a negative correlation between the two variables. The closer the dew point temperature was to the air temperature, the higher was the relative humidity of the environment. The rainfall values were very low (0.00±0.00-0.01±0.00) while the air pressure progressively increased from the month of March to June (991.28±0.02-994.74±0.03). There was no definite pattern for wind speed. The average Temperature-Humidity index (79.92) indicated that the birds were not within their thermal comfort zone.

Housing system effect on the thermo-physiological traits of Sasso hens is shown in Table 3. Rectal temperature was not significantly ($P>0.05$) affected by housing system. However, the mean values for pulse rate (144.32±0.55 versus 142.54±0.52), respiratory rate (31.91±0.42 versus 29.94±0.41) and heat stress index (1.76±0.03 versus 1.85±0.03) were significantly ($P<0.05$) higher in battery cage than the deep litter system.

Table 3. Effect of housing system on the thermo-physiological traits of Sasso laying hens

Parameters	Housing System	
	Deep litter (Mean±S.E.)	Battery Cage (Mean±S.E.)
Rectal temperature	41.62±0.01	41.61±0.01
Pulse rate	142.54±0.52^{b}	144.32±0.55^{a}
Respiratory rate	29.94±0.41^{b}	31.91±0.42^{a}
Heat stress index	1.76±0.03^{b}	1.85±0.03^{a}

S.E.: Standard error of means; ^{ab}Means within rows carrying different superscripts are significantly different ($P<0.05$)

There was divergent age effect on rectal temperature and pulse rate of Sasso layers. While birds that were 42 weeks old had higher rectal temperature (41.65±0.01 versus 41.58±0.01; $P<0.05$), their 52-week old counterparts had significantly higher pulse rate (142.24±0.53 versus 144.61±0.54; $P<0.05$) (Table 4). Respiratory rate and heat stress index were not significantly ($P>0.05$) influenced by age.

Table 2. Average values (Mean±S.E.) for meteorological indicators during the period of study

Parameters	Month				
	March	April	May	June	July*
Air Temperature (°C)	31.90±0.05	31.05±0.04	28.70±0.03	28.35±0.05	27.39±0.50
Dew Point (°C)	12.01±0.70	16.74±0.73	23.68±0.01	24.15±0.02	23.50±0.15
Relative Humidity (%)	43.04±0.17	60.51±0.18	75.39±0.13	78.85±0.18	80.31±2.27
Rainfall (mm)	0.00±0.00	0.01±0.00	0.01±0.00	0.01±0.00	20.92±5.63
Solar Radiation (W/m^2)	221.36±3.17	192.4±2.88	178.80±2.72	181.08±4.36	-
Pressure (mbar)	991.28±0.02	991.86±0.02	993.97±0.02	994.74±0.03	-
Wind speed (m/s)	0.83±0.01	1.20±0.01	1.03±0.01	0.51±0.26	4.65±0.32
Temperature-Humidity index	79.40	81.28	80.12	80.06	78.72

Source: Meteorological Unit, Faculty of Agriculture, Nasarawa State University, Keffi, Shabu-Lafia Campus, Nigeria; *NIMET (2017); S.E.: standard error of means

Table 4. Effect of age on the thermo-physiological traits of Sasso laying hens

Parameters	Age	
	42-week old (Mean±S.E.)	52-week old (Mean±S.E.)
Rectal temperature	41.65±0.01[a]	41.58±0.01[b]
Pulse rate	142.24±0.53[b]	144.61±0.54[a]
Respiratory rate	30.83±0.41	31.02±0.42
Heat stress index	1.81±0.03	1.79±0.03

S.E.: Standard error of means; [ab]Means within rows carrying different superscripts are significantly different (P<0.05)

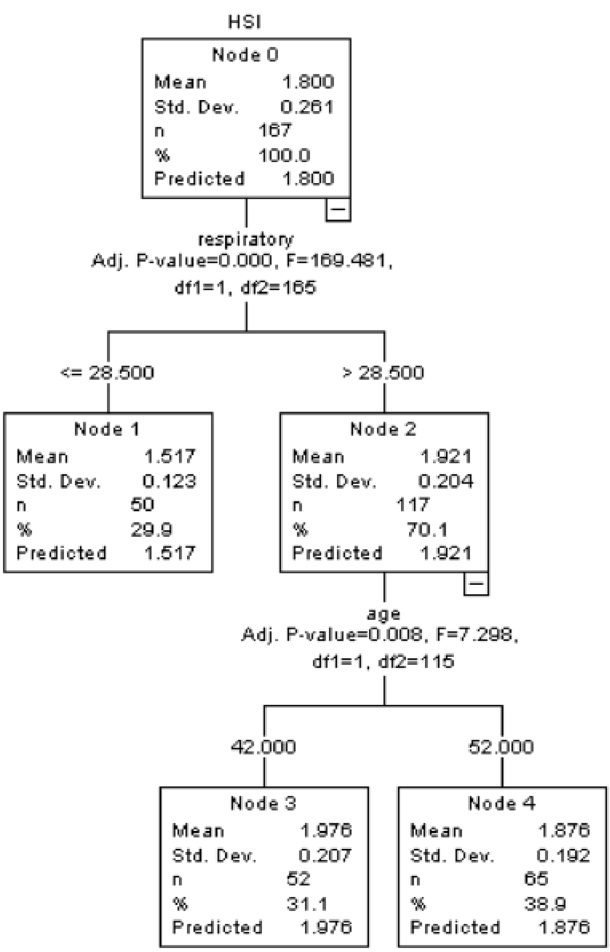

Figure 1. Regression tree showing respiratory rate and age as the most important parameters in predicting heat stress index

P–values of housing system and age interaction effect on rectal temperature, respiratory rate and heat stress index were significant (<0.001). The pairwise comparison showed that birds in cages appeared to be more stressed in terms of rectal temperature at week 42 than when they were 52-week old (Table 5). In terms of respiratory rate, 52-week old birds on deep litter appeared to experience more thermal discomfort, whereas in battery cage 42-week old birds were more prone to stress. Heat stress index followed a similar fashion. There was no significant (P= 0.269) housing system and age interaction effect on pulse rate.

The Exhaustive CHAID regression revealed five nodes (0, 1, 2, 3 and 4) (Figure 1). The root node (Node 0) reflects the descriptive statistics of heat stress index and was broken on the basis of respiratory rate into Node 1 and Node 2. Although Node 1 was homogenous (terminal node) as it was not divided into sub-groups, its mean value (1.517) for heat stress index prediction was less than that of Node 2 (1.921). Based on age, therefore, Node 2 was further divided into two terminal nodes (Nodes 3 and 4). The mean heat stress index of birds in Node 3 was predicted as 1.976 compared to 1.876 of those in Node 4. Both respiratory rate and age accounted for 53% of the variation in heat stress index of Sasso hens. Risk value (0.032) and Standard error (0.003) were low enough to validate the results.

.The mean values for PCV (P=0.925), Hb (P=0.925), RBC (P=0.955), WBC (P=0.738), MCH (P=0.248), MCHC (P=0.805) and MCV (P=0.187) did not significantly (P>0.05) vary between the deep litter and battery cage system (Table 6).

Table 6. Haematological traits of Sasso laying hens as affected by housing system at 52 weeks of age

Parameters	Housing System	
	Deep litter (Mean±S.E.)	Battery Cage (Mean±S.E.)
PCV (%)	27.00±4.80	26.25±5.95
Hb (g/dl)	8.95±1.61	8.70±1.98
RBC ($x10^{12}$/l)	2.55±0.49	2.60±0.53
WBC ($x10^{9}$/l)	7.10±0.82	6.54±1.36
MCH (pg)	35.33±0.68	32.83±1.83
MCHC (%)	33.15±0.11	33.12±0.05
MCV (fl)	106.88±2.24	98.53±5.14

S.E.: Standard error of means; No significant mean differences (P >0.05)

The mean values for cholesterol (3.10±0.09 versus 2.45±0.10; P=0.003) and glucose (9.95±0.40 versus 7.40±0.86; P=0.036) were significantly (P<0.05) higher in birds reared on deep litter compared

Table 5. Housing system and age interaction effect on the thermo-physiological traits of Sasso laying hens

	Deep litter		Battery cage		P[1]
	42-week old	52-week old	42-week old	52-week old	
Rectal temperature	41.61±0.02[a]	41.62±0.0[a]	41.69±0.02[a]	41.53±0.02[b]	<0.001
Respiratory rate	27.91±0.57[b]	31.97±0.58[a]	33.75±0.60[a]	30.08±0.60[b]	<0.001
Heat stress index	1.65±0.04[b]	1.87±0.04[a]	1.97±0.04[a]	1.72±0.04[b]	<0.001

[ab]Means within rows carrying different superscripts are significantly different (P<0.05); [1]P–values for Housing system and Age interaction

Table 7. Serum biochemical characteristics of Sasso laying hens as affected by housing system at 52 weeks of age

Parameters	Housing System	
	Deep litter (Mean±S.E.)	Battery Cage (Mean±S.E.)
Cholesterol (mmol/l)	3.10±0.09[a]	2.45±0.10[b]
Glucose (mmol/l))	9.95±0.40[a]	7.40±0.86[b]
Total protein (g/dl)	5.20±0.21[a]	5.30±0.15[a]
Albumin (g/dl)	3.23±0.24[a]	3.23±0.17[a]
Globulin (g/dl)	1.98±0.09[a]	2.08±0.12[a]

S.E.: Standard error of means; [ab]Means within rows carrying different superscripts are significantly different (P <0.05)

to those kept in cages. Other parameters such as the total protein (P=0.712), albumin (P=1.000) and globulin (P=0.518) contents were not significantly (P>0.05) affected (Table 7).

Discussion

The temperature-humidity index (average of 79.92) recorded in this study was above the threshold of 72 reported by Moraes et al. (2008). This indicates that the high ambient temperature as well as high relative humidity predisposed the birds to heat stress. The activity of the neuroendocrine system of poultry could be altered by high environmental temperatures, and this could affect thermoregulation and homeostasis, thereby resulting in heat stress. This has been reported as one of the most important environmental stressors having a negative impact on the productivity, immunity of laying hens (Lara and Rostagno, 2013) and the general wellbeing of birds (El-Tarabany, 2016).

Based on thermo-physiological traits, birds kept in cages appeared to be more stressed than their counterparts on deep litter. Increased respiratory rate could be attributed to a great demand for oxygen and the need for evaporative cooling (Molero, 2007). Caged birds are more susceptible to heat stress as a result of conductive heat loss in cages as birds are unable to seek a cooler place (www.hyline.com). Contrary to our findings, Barbosa Filho et al. (2005) reported that laying hens reared in the deep litter system had lower rectal temperature compared to those in cages. The significant difference between the two systems was attributed to the availability of greater space to hens on deep litter, which enabled good air circulation and permitted exchange of heat between the hens and the external environment.

Aluwong et al. (2017) revealed the significance of age as a determinant of thermal response of chickens to the stressful environmental conditions of the hot-dry season. As age increased, Sandercock et al. (2006) reported an increase in deep body temperature and panting caused by heat stress.

There is no clear cut difference in the response of birds kept in deep litter and battery cages at different ages as regards respiratory rate and heat stress index in this study as depicted in housing system and age interaction. It is possible that at 52 weeks of age, birds in the cage system have adapted to their new environment and have built enough immunity to adjust to the prevailing environmental conditions. However, Holt et al. (2011) reported that there is no general consensus as regards the superiority of one system over the other since many factors interplay to influence decision on the best housing system.

The small regression tree obtained in this study is informative and could easily be interpreted (Okoro et al., 2017) in terms of the relationship between heat stress index and respiratory rate as well as age of birds. This implies that at 42 weeks of age, birds with respiratory rate value of >28.500 gave a better prediction of heat stress index. Therefore, where information is lacking on heat stress index, it can be estimated from respiratory rate and the younger the birds, the easier the prediction. According to Song and Lu (2015), regression tree simplifies complex relationships between the response variables and predictor variables. The present findings may aid management practices while trying to curtail the detrimental effect of heat stress.

The present values obtained on heat tolerance traits indicate the genetic potential of Sasso as tropically adapted birds. This is congruous to earlier submissions on the genetic basis of heat stress response (Soleimani et al., 2011; Felver-Gant et al., 2012; Rojas-Downing et al., 2017); which include single genes of major effect and complex multigenic control (Lamont et al. 2014). The possession of large combs by Sasso birds (subjective evaluation) could be an adaptive feature that might function as a biological heat exchanger. This is in consonance with the report of Gerken et al. (2006) that the comb might play a role in heat dissipation, thereby facilitating evaporative cooling of the brain; a device to maintain thermal homeostasis when birds are exposed to high environmental temperature.

The non-significance difference in the PCV, Hb, RBC, WBC, and total proteins values in both housing systems is a pointer that neither the deep litter nor the battery cage system negatively interfered with homeostatic mechanism. This is consistent with the findings of Pavlik et al. (2007). Similarly, Davis (2010) reported that birds in cages and those on floor pens were not significantly different in stress indicator (average heterophil/lymphocyte ratio). The present biochemical indices may provide useful information on the immune status of bird and could also be parameters of consideration while planning genetic improvement programmes (Ladokun et al., 2008; Panigrahy et al., 2017).

The non-significant values recorded for total protein and albumin in both housing system are congruous to the submission of Yang et al. (2014). The higher cholesterol content of birds on deep litter may be attributed probably to higher feed consumption, which complements its endogenous synthesis in hens. The average value 3.10 mmol/l (55.80 mg/dl) obtained in this study falls within the normal reference values of 52.00-148.00 mg/dl (Mitruka and Rawnsley, 1977). Therefore, it does not constitute any health challenge and may not distort the physiological adaptation mechanism of the birds. More so that birds on deep litter due to physical activity such as mobility has the propensity to convert excess serum cholesterol to egg cholesterol. It has been reported that a considerable amount of serum cholesterol is deposited in the egg during the process of catabolism (Kurtoglu et al., 2004). The present findings are similar to the submission of Zemkova et al. (2007) that researched housing system altered yolk and egg cholesterol concentration, which was lower in the cage system and higher in the deep litter system. However, the latter focused on egg as compared to plasma concentration in the former. In contrast to our findings, Yang et al. (2014) reported that total cholesterol did not significantly vary between birds on deep litter and those in cages (3.50 ± 0.712 versus 3.82 ± 1.13). In the same vein, Pistekova et al. (2006), Küçükyılmaz et al. (2012) and Alabi et al. (2015) reported that housing system did not influence cholesterol concentration in laying hens. The higher glucose content of birds reared on deep litter may be attributed to effective carbohydrate metabolism as a result of increase in glucocorticoids secretion (Nidamanuri et al., 2017) that regulates glucose homeostasis. However, Lay Jr et al. (2011) reported that each housing system had its own peculiar challenges as regards the stress it imposes on hens. Therefore, care must be taken while trying to switch to an alternative housing system

Conclusion

Sasso birds exposed to thermal discomfort exhibited varying degrees of response to heat stress under the deep litter and battery cage system and at age 42 and 52 weeks, respectively. Heat stress index was better predicted from respiratory rate and at 42 weeks of age. The present information may guide appropriate management practices to ameliorate the detrimental effect of heat stress in the tropics. However, subsequent studies may exploit the possibility of considering data collection at more phases of production of Sasso birds.

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