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Productive and Physiological Responses of Heat-Stressed Dominant Black Hens to Artificial Ventilation and Modified Layers Mash in Nigeria

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Abstract: One thousand 53 weeks old heat stressed dominant black hens at 82% egg production were allotted into four treatment groups and used to determine the effects of ventilation and modified Layer's mash (MLM) on productive and physiological parameters. Treatment 1 (T1) are birds housed with fan and fed layers mash. Treatment 2 (T2) are birds fed MLM with fans, treatment 3 (T3) were fed layers mash but no fans. Treatment 4 (T4) fed MLM but no fans. Data were subjected to percentages and two way ANOVA. T1 had the highest mean feed intake (114.56 ± 0.035 gm) and T4 had the lowest consumption (107.87 ± 0.086 gm). The highest mean weekly mortality (11.10 ± 0.98) was recorded in T3 and the lowest 6.31 ± 0.21 in T2. T2 had the highest mean % egg production (78.18 ± 2.80) and the lowest percentage (68.37 ± 3.10) in T3. The result of this study also revealed that both relative humidity (RH) and temperature correlated negatively and significantly with feed consumption across the treatments. The PCV of T1 (37.20 ± 0.70) is equal to T2 (37.40 ± 0.10), but significantly greater ($p > 0.05$) than T3 (34.45 ± 0.05) and T4 (34.20 ± 0.50), the WBC followed same trend, while other haematological parameters show no significant differences. T2 has the most significant increase in serum globulin and Uric acid, followed by T1, all other parameters are not significantly different. The electrolytes (K^+ , Na^+ , Ca^{2+} , Cl^- and HCO_3^-) also followed the same trend. T2 has the best performance, therefore to alleviate the effects of heat stress, dietary modifications and good ventilation is recommended.

Key words: Artificial ventilation, electrolytes, haematology, heat stress, percentage egg production, serum biochemistry

INTRODUCTION

Environmental stressors, such as heat stress, are particularly detrimental to animal agriculture (Nienabar and Hahn, 2007; Nardone *et al.*, 2010; Renaudeau *et al.*, 2012). The issue of environmental stress has quickly become a great point of interest in animal agriculture, particularly due to public awareness and concerns. The importance of animal responses to environmental challenges applies to all species. However, poultry seems to be particularly sensitive to temperature-associated environmental challenges, especially heat stress. It has been discovered that modern poultry genotypes produce more body heat, due to their greater metabolic activity (Settar *et al.*, 1999; Deeb and Cahaner, 2002) as a result of the high rate of egg production (Blem, 2000). Hens lack sweat glands and their respiratory water evaporation rate is not high enough to maintain normo-thermia at high ambient temperatures and relative humidity (Dawson and Whitlow, 2000).

The two most important weather variables that have direct bearing on birds' activity are temperature and relative humidity. Research evidence had shown that throughout the whole range of practical environmental temperature, laying hens have physiological responses that affect their productive performance (Keener *et al.*, 2006). Irshad *et al.* (2012) posited that it is not the degree of heat alone that causes distress to animals in the tropics but its combination with humidity and the duration of these conditions.

Environmental temperature in South-Western Nigeria is often higher than $18-21^\circ\text{C}$ (Charles, 2002) recommended for optimal productivity in laying chickens (Abioja, 2010). Often, growth, performance and welfare of the birds are compromised (Shane, 1988; Altan *et al.*, 2000; Hai *et al.*, 2000; Abu-Dieyeh, 2006) and survival lowered (Brake, 1987) because of the birds responses to the stressor.

The relationship between ambient temperature and performance had been studied extensively in poultry, Olawumi and Ogunlade (2010) documented significant negative correlations between egg production and high temperature in layer breeders. In ostrich, Rozenboim *et al.* (2007) reported that egg production and weight decreased from naturally and experimentally high temperature. Previous researchers have documented that year, season and month of production have direct bearing on production and reproductive performance of animals (Chowdhury *et al.*, 2004). Seasonal influence on egg production (Bawa *et al.*, 2001; Olawumi, 2011), fertility and hatchability (Olawumi, 2007) and mortality (Bawa *et al.*, 2001; Olawumi, 2007) had been documented in literatures. Some of the negative and indirect effects of high temperature on birds' performance are reduced feed intake (Njoya and Piccard, 1994) and declining immune response (Dauda *et al.*, 2006) of the birds to invading pathogens. Post *et al.* (2003) reported that high environmental temperature leads to excretion of some minerals like Ca, Fe, Zn, thereby resulting in decreased bone strength and thin shelled eggs.

Heat stress in birds can lead to decreased feed intake, higher water intake (Fasanmi and Sansi, 2009), increased respiratory rates (panting), diarrhoea (flushing) and decreased activity. These responses result in lower egg production, smaller egg weights, respiratory alkalosis and decreased immune function. Respiratory alkalosis reduces the blood's ability to transport calcium resulting in losses of shell quality and reduced bone strength (Gingerich, 2012).

Stress responses are considered to be essentially adaptive or protective and thus should prevent or minimize detrimental effects of the stressor to some extent, but these adaptation and protection can be further complemented or aided through some ameliorative measures. Hence the need for this work, to determine and compare the temperatures and relative humidities of the pen houses with or without artificial ventilation (fans) in hot humid weather. Also, to evaluate and compare performance parameters and physiological responses of heat stressed hens fed modified layers mash and exposed to fans.

MATERIALS AND METHODS

Experimental location: The research study was carried out at a Commercial Poultry Farm in Abeokuta, Ogun State, located at (latitude 7°13' 47.36" N; longitude 3°24' 11.98" E). This zone has an average annual maximum and minimum temperature of 31.8±3.2°C and 18.0±3.7°C, respectively. The monthly average rainfall during the rainy season (May-October) is 148±68.4 mm (69.2-231.9 mm), while the monthly relative humidity is 71.1±9.7%. The zone is characterized by three seasons: Harmattan (November-February), hot-dry (March-May)

and rainy (June-October) seasons (Igono *et al.*, 1982, Ayo *et al.*, 1999).

Experimental design: One thousand 53 weeks old dominant black hens at 82% egg production, housed in battery cages located in an open sided pen houses were used for this research work. One thousand laying hens were allotted into four treatment groups by complete randomize design viz., treatment 1 (T1) comprised of birds exposed to fan and fed on layer mash (feed 1), birds in treatment 2(T2) comprised of birds exposed to fan and fed on modified layer mash (feed 2), Treatment 3 (T3) had birds fed on layers mash had no fan. Birds in treatment 4(T4) group were kept in a house without fan and fed on modified layers mash. Each treatment was replicated five times and each replicate had fifty hens. The birds were housed in a battery cage with an individual space of 400 cm². The birds housed in environmentally controlled houses were exposed to fan (OX fan) from 6:00 to 17:00 h daily for a period of nine weeks. The two types of feed used in this study were formulated using commercial feed formulation software and compounded to meet the nutritional requirements of the hens and the prevailing climatic condition. The modified feed (F2) is derived by supplementing the layers mash (F1) with 1% vitamin C, 0.5% choline, chloride and 1.2% limestone.

Meteorological parameter data collection: The daily mean ambient temperatures and relative humidity of the various pens (treatments) were monitored throughout the experimental period. The temperatures were taken using mercury in glass thermometer, while the relative humidity were determined using the hygrometer (wet and dry bulb thermometers).

Other data collection: Parts of the data collected from farm records and used for this study include percentage egg production, mortality, T°C, RH and Feed Intake (FI) from November to December, 2014. Thereafter, the percentage production and mortality were monitored and recorded on daily basis, while average percentage production and total mortality were calculated on weekly basis.

Blood collection and laboratory analysis: Blood collection and evaluation was done following 16 weeks of the research at age 68 weeks, blood samples were collected from 10 birds per replicate through wing vein into 2 sets of well labeled sample bottles. One set of sample bottles contained anticoagulant, ethylene diamine tetra acetic acid (EDTA) for haematological study and the second set without EDTA. Blood samples were collected, kept on ice in a cooler and transferred to the Anatomy and Physiology laboratory of Federal College of Animal Health and Production Technology,

Moor Plantation, Ibadan. These samples were immediately used for determination of haematological parameters, Haemoglobin concentration was determined spectrophotometrically, as described by Franceschini *et al.* (1997), Packed cell volume (PCV) and Red Blood Cell (RBC) counts were determined as described by Dacie and Lewis (1991). Total White Blood Cell (WBC) counts were determined using Neubauer haemocytometer. Blood constants (Mean Corpuscular Volume, Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentration) were determined using appropriate formulae as described by Jain, in Veterinary haematology (Schalm *et al.*, 1986). The second sets of samples without EDTA were centrifuged and serum decanted for serum biochemical and electrolyte analysis. Total serum protein was determined using appropriate kits with basic procedure of Kohn and Allen (1995), albumin was measured using bromocresol green (BCG) binding technique as described by Abdul Hameed (2012), while serum cholesterol was determined by the Roschlan methods, Uric acid in the blood was determined by method of Gabbey (2012). The electrolytes (sodium, potassium, chloride and bicarbonate) were determined and analyzed, by using IDEXX Vetlyte[®] Electrolyte and blood gas analyzer.

The proximate composition of the Layer's mash was determined according to the official method of analysis (AOAC, 1995).

Statistical analysis: The productive performance (egg production) and mortality (dead birds) were expressed as percentage of total number of hens per treatment. All haematological and serum biochemical data from the laboratory were subjected to two way Analysis of Variance (ANOVA) and errors were calculated as Standard Errors of Means (SEM). Significant treatment means were compared using Duncan's New Multiple Range Test as outlined by Obi (1990), significance was accepted at the 0.5 level of probability.

RESULTS

Table 1 shows the percentage ingredient composition and the proximate analysis of both the layers mash (F1) and the modified layers mash (F2). The metabolizable energy (ME) level of the F2 diet while comparably lower to the F1 diet has a higher inclusion rate of micronutrients like choline chloride, vitamin C, calcium, lysine and methionine

The result of this study has shown in Table 2 that there is a significant difference in the mean feed intake across the four treatment groups. Birds in (T1) have the highest mean feed intake of 114.43±0.035 and those in group T4 have the least meant feed intake of 107.87±0.037 g. This study has also shown (Table 3) that the week of study has a significant effect on the mean feed intake

Table 1: Feed composition (Layer's and modified Layer's mash)

	Feed 1 Quantity (kg)	Feed 2 (modified) Quantity
Ingredients (kg/1000 kg)		
Maize	520	520
Soya meal	100	100
Ground nut cake	100	100
Fish meal (72%)	20	40
PKC	40	0
Wheat offal	106	118
Bone meal	20	20
Limestone	85	90
MycotoXin binder	1	1
Lysine	1	2
Methionine	1	2
Salt	3	3
Vitamin + Mineral Premix	3	3
choline chloride	0	0.5
Vitamin C	0	1
Total	1000	1000
Composition (proximate analysis) %		
Dry matter	93.6	94.5
Crude protein	16.3	17
Ether extract	3.8	3
Crude fibre	3.4	3.5
Ash	4.3	4.5
Nitrogen free extract	65.8	66.5
Composition (calculated)		
Metabolizable energy (Kcal/kg)	2653.14	2609.42
Crude protein	17	18
Fat	4.2	3.8
Fibre	3.3	3.2
Calcium	3.75	3.9
Available phosphorus	0.41	0.43
Methionine	0.39	0.56
Lysine	0.95	1.1

($p < 0.001$). The highest level of feed intake was obtained in week 1. There was no difference in mean feed intake observed in the first four weeks but was significantly more than the feed consumed in the other weeks of the study. There was however no significant difference in feed intake of birds in weeks 7 and 8, weeks 9 and 11 and weeks 14 and 15.

A significant interaction effect was also observed between the weeks of study and the treatment groups on one side and the feed intake of the birds on the other.

The result of the Pearson correlation study also revealed that both the relative humidity (RH) and temperature correlated negatively and significantly with feed consumption across the treatment groups. This observation was made throughout the weeks of study.

In (T1), Table 4 there was a significant negative correlation between the RH, temperature ($r = -0.55$, $p < 0.05$; $r = -0.58$, $p < 0.05$) and feed intake. In the T2 group, a feed intake had negative but insignificant correlation with both temperature and RH ($r = -0.50$, $p > 0.05$; $r = -0.44$, $p > 0.05$), throughout the weeks of study. The correlation between RH and temperature with feed intake was also significant in the T3 ($r = -0.88$, $p < 0.01$;

Table 2: Mean ± SEM of mean percentage egg production, mortality and feed consumption of birds kept under different housing and feed regime

Treatment groups/production indices	T1	T2	T3	T4
Mean % egg production	75.20±1.10 ^a	78.18±1.12 ^b	68.37±1.08 ^c	72.68±1.22 ^d
Feed consumption(g)	114.56±0.035 ^a	113.43±0.076 ^b	109.68±0.02 ^c	107.87±0.086 ^d
Mean weekly mortality	7.68±0.004 ^a	6.31±0.001 ^b	11.00±0.09 ^c	9.37±0.06 ^d

Values along with the same row with different superscript are significantly different, T1: Birds exposed to fan and layers mash, T2: Birds exposed to fan and modified layers mash, T3: Birds exposed to no fan and layers mash, T4: Birds exposed to no fan and modified layers mash

Table 3: Weekly variation in mean % egg production, feed consumption and mortality

Weeks	Mean % egg production	Mean weekly feed intake(g)	Mean weekly mortality
1	82.25±0.03	120.00±0.87 ^{ab}	6.50±0.05
2	82.75±2.34	120.00±0.68 ^{ab}	5.75±0.02
3	82.25±3.09	120.00±0.84 ^{ab}	5.75±0.02
4	82.25±4.50	120.00±0.77 ^{ab}	5.25±0.04
5	79.50±0.03	117.00± 2.34	15.50±0.11
6	77.75±0.041	114.25±1.10	13.00±0.14
7	74.25±0.056	105.00±1.78 ^a	13.50±1.34
8	72.25±0.054	105.00±1.76 ^a	12.25±1.20
9	67.75± 0.431	106.75±1.10 ^b	9.50±0.25
10	66.50±0.040	107.25±1.10 ^b	8.50±0.06
11	67.00±0.032	106.75±1.12	7.75±0.34
12	66.75±0.036	108.50±1.10	7.25±0.05
13	67.75±0.08	108.25±2.30	7.25±0.07
14	68.50±0.04	108.00±1.11 ^c	6.50±0.045
15	69.75±0.03	108.00±1.21 ^c	6.25±0.24
16	70.75±0.10	107.00±3.10	7.00±0.003

All the value under the mean % egg production and the mean weekly mortality are significantly different. Values in the middle column with same superscripts are not significantly different.

r = -0.93, p<0.01) and T4 group(r = -0.88, p<0.01; r = -0.95, p<0.01). The correlation indices were stronger in T3 and T4 compared to T1. Table 4 also revealed that both temperature and humidity in T3 and T4 groups are higher compared to groups T1 and T2.

The result of this study as shown also revealed that mean daily mortality was significantly affected by the factors of treatment and week of study. There was also a significant interaction between treatment and week of study. The highest mean daily mortality of 11.10±0.98 was recorded in group T3 and the lowest 6.31±0.21 in T2 (Table 3). The highest mean daily mortality of 15.50±0.67 was recorded in week 5 while the lowest mean weekly mortality rate of 5.25±0.54 was recorded in week 4 (Table 3). There is also a significant difference in the weekly mortality.

Table 3 also showed that mean % egg production was affected by the treatment group and week of study. There was also significant interaction between treatment groups and weeks of treatment. Birds in the T2 group had the highest mean % egg production of 78.18±2.80 and the lowest egg production value of 68.37±3.10 were recorded in T3 (Table 2). There was a significant difference in the mean % egg production recorded during different week of study (Table 3).

The haematology result in table 5 showed that T2 birds had the highest PCV compared to other treatment group while birds in T4 had the lowest mean PCV value of 34.20±0.14. There is a significant difference in PCV level of birds in T1, T3 and T4. There is however no significant

difference in the PCV of T1 and T2. There is also no significant difference between T3 and T4. The WBC count of birds in T2 was significantly higher than in all other groups studied. The WBC count of T3 and T4 groups were not significantly different, their respective WBC values are significantly lower than those of T1 and T2. All other hematological parameters were not significantly affected by the treatment groups.

Table 6 showed the result of the biochemical studies. The globulin, sodium, potassium, calcium, chloride, bicarbonate level of T2 was significantly higher than in all the treatment groups. Except for glucose, T2 has the highest values for all the parameters. The glucose level of the T3 and T4 are not significantly different. The glucose level of birds in T4 group was the highest and that of T2 group was the lowest. There is however no significant difference in the Total protein, albumin and uric acid level of all the other treatment groups.

DISCUSSION

This study has shown that birds exposed to fan and fed modified layers mash (T2) had the highest % egg production and the lowest mortality rate. It also showed that birds fed with layers mash without fan had the highest mortality. Feed consumption in birds fed with MLM is also observed to be lower. This may be because modified layers mash is formulated to help the hen during heat stress and to reduce adverse effects of the heat on the hen's performance and other body physiology. The feed has Metabolizable Energy (ME)

Table 4: Showing the Mean±SEM of temperature, relative humidity, feed intake and the correlation indices of birds kept under different housing and feeding conditions

Treatment groups	Relative humidity			Temperature			Feed intake (g)
	x	r	p	x	r	p	
T1	69.31± 2.52	-0.55	0.02	32.15±0.62	-0.58	0.01	114.56±4.25
T2	69.25± 2.56	-0.44	0.08	32.12±0.62	-0.50	0.05	113.43±4.93
T3	73.50± 3.79	-0.88	0.01	34.12±1.89	-0.93	0.01	109.69±7.33
T4	73.56±3.68	-0.88	0.01	34.12±1.89	- 0.95	0.00	107.87±8.95

T1: Birds exposed to fan and layers mash, T2: Birds exposed to fan and modified layers mash, T3: Birds exposed to no fan and layers mash, T4: birds exposed to no fan and modified layers mash. X: Mean ± SEM, r: correlation index, p: p value

Table 5: Showing the haematological indices of birds kept under different housing and feeding regime

Parameters	T1	T2	T3	T4
PCV(%)	37.20±0.14 ^a	37.40±0.84 ^a	34.45±0.21 ^c	34.20±0.14 ^c
Hb(g/dl)	13.45±0.07	14.05±0.07	13.30±0.28	12.80±0.14
RBC X10 ⁶ /µl	3.08±0.04	3.40 ±0.00	3.20±0.07	3.10±0.00
WBC X10 ³ /µl	21.10±0.00 ^a	23.35±0.21 ^a	18.60±0.14 ^{ab}	19.95±0.07 ^{ab}
MCV (fl)	120.79±2.12	110.00±2.49	107.67±1.71	110.32±0.45
MCH (pg)	43.67±0.36	41.32±0.20	41.57±0.04	41.29±0.45
MCHC (g%)	36.15±0.33	37.58±1.04	38.60±0.58	37.42±0.26

Values are expressed as Mean ± SD. Values on the same row with different superscript are significantly different, P<0.05

Table 6: Showing the biochemical indices of birds kept under different housing and feeding regime

Parameters	T1	T2	T3	T4
TP (g/dl)	3.55±0.07	4.45±0.07	3.45±0.07	3.60±0.07
ALB (g/dl)	2.60±0.00	3.10±0.14	2.85±0.07	2.95±0.07
GLOB (g/dl)	0.95±0.07 ^d	1.35±0.07 ^a	0.60±0.00 ^{ab}	0.65±0.14 ^c
CHOLE (g/dl)	132.50±3.53	130.00±7.07	141.00±1.41	135.00±2.82
GLUCOSE (g/dl)	207.50±3.53 ^{ab}	202.50±3.53 ^b	214.00±5.65 ^a	217.50±3.53 ^a
K ⁺ (Meq/L)	4.83±0.25 ^d	5.82±0.02 ^a	3.17±0.02 ^{ab}	3.58±0.02 ^c
Na ⁺ (Meq/L)	136.85±6.15 ^d	154.70±0.42 ^a	121.25±1.06 ^{ab}	129.38±0.87 ^c
Ca ²⁺ (mg/dl)	29.50±0.70 ^d	36.25±0.35 ^a	22.75±0.35 ^{ab}	28.15±0.49 ^c
HCO ₃ ⁻ (Meq/L)	12.05±0.07 ^d	16.10±0.28 ^a	10.85±0.49 ^{ab}	10.35±0.07 ^c
Cl ⁻ (Meq/L)	143.50±2.12 ^d	166.50±2.12 ^a	122.50±3.53 ^{ab}	130.25±6.71 ^c
URIC ACID (g/dl)	1.90±0.14	2.60±0.28	1.05±0.07	1.18±0.11

Values are expressed as Mean ± SD. Values on the same row with different superscript are significantly different, P<0.05

which is slightly reduced when compared with F1, because if the ME is high during metabolism lot of heat will be generated that will affect the hen negatively (Moreki, 2008). The amino acids (lysine and methionine) which are readily available (Moreki, 2008; Chandrasekar, 2011), were increased in F2. Also choline chloride was added to aid the mobilization of liver and abdominal fat (Wasserman, 2015) and indirectly involved in the reduction of the temperature of the hen.

The calcium (Ca) source was increased in F2 so as to help in the bone strength and shell quality, because the absorption of Ca is impaired during heat stress (Fasanmi *et al.*, 2014). Vitamin C was added to F2 to reduce heat stress in the laying hens, because it helps in inhibiting the secretion and release of corticosteroid, which may be cytolytic at high concentration during stress episodes (Sahin *et al.*, 2003; Mahmoud *et al.*, 2004; Sobayo, 2005; Mbajjorgu *et al.*, 2007).

This study has also revealed that birds kept in houses without fan experienced a more humid and hotter environment and their feed consumption was adversely affected by these conditions. This observation has been reported by other workers. The adverse effect of high

temperature and humidity were lesser in birds housed with fan and fed modified layers mash. These birds, although did not eat as much as the birds housed with fan and fed layers mash, their mortality rate was lower and their feed consumption was better than any other group. The modified layers mash is denser than the layers mash and this is probably the reason for the reduced feed intake.

Haematological parameters of heat stressed hens exposed to fans and or modified layers mash, show significant differences in only two parameters out of the seven measured. The PCV was higher (p>0.05) for treatments T1 and T2 whose pens were fitted with fans when compared with T3 and T4 whose pens have higher RH and T°C (see Table 2), this is attributable to heat and functional stress which impaired the synthesis of blood cells in birds (Oladele *et al.*, 2003), also due to excessive excretion of Fe²⁺ (Post *et al.*, 2003). Whether fed on modified layers mash or layers mash, birds kept in houses with fan had a higher PCV. This may be due to the ameliorative effect of the fan on prevailing adverse weather conditions in these pen houses. The presence of fan allows for feed intake and efficiency. This may

allow for better nutrient utilization and hence enhanced erythropoiesis. The higher level of WBC count of birds in T1 and T2 may also be attributed to the same reason. Birds whose houses are not fitted with fan appeared to be immuno compromised. The low level of WBC in these birds laid credence to this assertion.

The higher feed intake of birds kept in houses with fan also may have accounted for the higher level of serum electrolytes and globulin. These electrolytes are low because they are lost during panting, diarrhea and respiratory alkalosis during heat stress (Gingerich, 2012). The drop in Ca^{2+} level in T3 (22.75 ± 0.25) and T4 (28.15 ± 0.35) when compared with other treatments is as a result of increased carbon dioxide levels and higher blood pH (i.e., alkalosis), which in turn hampers blood bicarbonate availability for egg shell mineralization and induces increased organic acid availability, also decreasing free calcium levels in the blood, due to inability of blood to transport it, this is further reflected in the quality of shell and percentage egg production (Marda and Arad, 1989). The decreased protein intake experienced by the birds whose houses are not fitted with fans would lead to hypoproteinemia generally and specifically hypoglobulinemia (McNadabb and King, 1993; Sahin *et al.*, 2001). This will lead to a fall in level of immunoglobulin (antibodies) leading to declining immune response (Dauda *et al.*, 2006; Obidi *et al.*, 2008) and birds will become diseased easily (Obidi *et al.*, 2008; Ayo *et al.*, 2010; Fasanmi, 2011).

The higher level of serum glucose and lower level of serum protein seen in birds kept in houses without fan compared to the birds kept in houses with fan agrees with the findings of Sahin *et al.* (2001) and Olanrewaju *et al.* (2006) that high level of plasma glucose observed in heat stressed birds is as a result of increased gluconeogenic activity of corticosteroid produced.

Conclusion: In terms of egg production, feed intake and mortality rate, birds exposed to fans and adjusted layers mash performed better than birds in other treatment groups studied. Therefore to alleviate the effects of heat stress, dietary manipulations and good ventilation are necessary as these can help reduce metabolic heat production, reduce ambient temperature, maintain nutrient intake and reduce mortality rate.

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