NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 50, 2017 (1): 15–20

ISSN 1337-9984

RECTAL TEMPERATURE, HEART RATE, PACKED CELL VOLUME AND DIFFERENTIAL WHITE BLOOD CELL COUNT OF LAYING PULLETS TO HONEY SUPPLEMENTED WATER DURING HOT – DRY SEASON

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ABSTRACT

Honey has medicinal properties which may help ameliorate adverse effects of heat stress in laying chickens. It has antimicrobial, antibacterial and anti-oxidant properties. This study investigated the use of honey in drinking water as a natural alternative to help alleviate heat stress in laying pullets during hot-dry season (December – April) in the tropics. One hundred and twenty *Isa Brown* layers at 28 weeks old weighing 1.5 ± 0.1 kg were used in a 16 week experiment. The birds were randomly allotted to 3 treatments of 4 replicates containing 10 birds each. The treatments were 0 ml honey per 1 L of water (0 H), 10 ml honey per 1 L of water (10 H) and 20 ml honey per 1 L of water (20 H). Data on heart rate, rectal temperature and haematological parameters were collected and subjected to one way analysis of variance. Rectal temperature was significantly ($p < 0.05$) higher in honey treated groups compared to control group. However, heart rate was significantly ($p < 0.05$) lower in 20 H birds (300.9 ± 1.70 bpm) compared to 0 H birds (313 ± 1.70 bpm). Honey supplementation did not significantly ($p > 0.05$) affect packed cell volume, heterophil, monocyte, eosinophil and heterophil-lymphocyte ratio. Birds given 20 H had a significantly ($p < 0.05$) higher lymphocytes count (50.6 ± 0.79 %) while birds that received 10 H had a significantly ($p < 0.05$) lower basophil count (3.1 ± 0.39 %). These findings indicate that the use of honey supplemented in drinking water reduced HR and basophil count.

Key words: heat stress; honey; layers; temperature; haematology

INTRODUCTION

Hot-dry season in the tropical environment is characterized by high environmental temperature, which sometimes exceeds 30 °C (Abioja *et al.*, 2011). Exposure of laying birds to high ambient temperature in poultry houses in the tropical zones elicits a series of responses in laying chickens which is generally termed heat stress (Ayo *et al*., 2011). It is caused by high ambient temperatures that exceed the thermoneutral zones of poultry species and when coupled with high humidity has a detrimental effect on commercial broiler and layers (Nienaber and Hahn, 2007). Heat stress occurs mainly in the hot – dry season and during this period, birds have limited physical resource (nutrient) for growth and reproduction in response to environmental change and voluntary feed consumption is drastically reduced (Khan *et al*., 2011). The adaptation to this new challenge requires redistribution of body reserve of energy and protein to thermoregulation at the cost of decreased growth and reproductive efficiency (Puron *et al*., 1994; Kadim *et al*., 2008).

Most poultry farmers make use of commercial and synthetic anti-stress and anti-oxidants to help chickens cope with heat stress. Alternatives to the use of chemicals such as anti-stress, anti-oxidants and antibiotics lie in discovery and proper utilization of natural plant materials and extracts that have the necessary properties needed

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Received: July 7, 2016
Accepted: August 11, 2016

(Bedford, 2000; Wenk, 2003; Vidanarachchi *et al.*, 2005; Ramnath *et al.*, 2008; Zhang *et al*., 2009; Ali *et al*., 2010). Various efforts have been geared towards exploration of these materials and one promising source of natural anti-stress/anti-oxidant is honey (Estevinho *et al.*, 2008; Mohamed *et al*., 2002; Gheldof *et al*., 2003; Aljadi and Kamaruddin, 2004; Wasagu *et al.*, 2013).

MATERIALS AND METHODS

Experimental location and meteorological observations

This experiment was carried out at Aiyedoto Farm Settlement, Ojo Lagos, Nigeria (latitude 6°27'25"N, longitude 3°12'21"E and altitude of 36 meter above sea level). The climate of the experimental site is humid, located in the rain forest vegetation zone of western Nigeria. Wet-and dry-bulb temperatures and relative humidity at the level of the birds in the pen at 8:00 and 16:00 h were monitored throughout the experimental period. The temperature-humidity index was calculated from relative humidity and wet- and dry-bulb temperature data.

Experimental animals and management

ISA Brown layer chickens (n = 120; aged 28 weeks) were used for the experiment for a period of 16 weeks. The birds were randomly allocated to three (3) treatments consisting 4 replicates and 10 layer birds per replicate. Standard ration was given *ad libitum* to the birds. The birds were offered honey supplemented water throughout the experimental period.

Data collection

Rectal temperature and Heart rate measurements

The rectal temperature (RT) of 3 birds randomly picked from each replicate was measured using digital thermometer (0.1 °C accuracy). It was inserted into the rectum and held until it beeped and the RT was read off on the visual display unit as described by Yahav and McMurtry (2001). The monitoring of RT started from week 7 to the end of the experiment due to unforeseen circumstances. The use of a stethoscope placed on the chest region of the birds was used to monitor the heart rate (HR) per minute.

Haematological parameters

Packed Cell Volume determination

Blood sample was collected from one randomly picked bird from each replicate at the end of the experimental period via brachial vein using 2 ml sterilized syringes (23 G) and needles into EDTA bottles. Haematocrit value was measured by using micro-haematocrit method according to Crawford *et al*. (1987). Blood samples were drawn into micro-haematocrit tube and one end of the tube was blocked with paraffin wax. The tubes were arranged in a micro-haematocrit centrifuge and centrifuged at 4000 rpm for five minutes. The proportion of packed blood cells was read off on micro-haematocrit reader.

Differential count determination

For differential leucocyte counts, blood smears were made on glass slides. The smears were stained with Wright stain for 15 minutes. Leucocytes differentials (heterophils, lymphocytes, eosinophils, monocytes and basophils) were counted from each smear with the aid of a microscope. The heterophil-lymphocyte ratio (H:L) was calculated by dividing the number of heterophils by the number of lymphocytes.

Statistical analysis

Data collected were subjected to one way analysis of variance (ANOVA) using SYSTAT (1992) using the model: $Y_{ij} = \mu + T_i + \sum_{ij}$; where Y_{ij} = dependent variables, μ = population mean, T_i = i^{th} effect due to addition of honey to drinking water (i = 1, 2 ,3), and $\sum_{ij}$ = residual error. Means that are statistically ($P < 0.05$) different were separated with Duncan multiple range test (DMRT).

RESULTS AND DISCUSSION

Table 1 shows the meteorological variables obtained in this study. The values were above the thermal comfort zone for egg laying chickens which is 16 - 25 °C (Sahin *et al.*, 2006) in the tropical regions. The average temperature recorded during this experiment was 31.5 °C, about 6.5 °C above the upper critical limit.

Table 1: Average daily values for meteorological parameters observed during the experimental period

	8:00 h	16:00 h	Average
Dry-bulb temperature (°C)	30.4	32.5	31.5
Wet-bulb temperature (°C)	27.8	28.8	28.0
Relative humidity (%)	82.3	76.7	79.5
THI	83.8	86.0	84.9

The combination of average ambient temperature and average relative humidity of 79.50 % gave an average temperature-humidity index of 84.92 which is higher than the THI threshold of 70 established for poultry (Tao and Xin, 2003; Karama *et al.*, 2007) which is a clear indication that the laying chickens were heat stressed.

It could be observed that layers given 20 H had lower rectal temperature than 0 H layers in the early stages of honey administration and later a reverse case as the 0 H layers had lower RT (Table 2). Layers that received 10 H had higher RT (41.13 ± 0.033 °C) than the control group (40.68 ± 0.032 °C). This is in contrast to Osakwe and Igwe (2015) who reported a mean lower RT with the administration of honey in the drinking water of the laying birds. This shows that the antioxidant effects of honey helped relieve the imbalance caused by oxidative stress due to the lack of antioxidant capacity that could be caused by abundant ROS from an environmental or behaviour stressor (Tatli *et al*., 2012) which in this case is heat stress for a certain period of time before causing a negative effect to the laying birds. The increase in temperature could be as a result of increased feeding which increases body temperature via digestion. Njoku and Nwazota (1989) demonstrated that adding ascorbic acid in the feed formula improved feed intake and feed utilisation.

Table 2: Effect of honey supplementation on rectal temperature (°C) of laying chicken during hot-dry season

Week	0 H	10 H	20 H	P-value
7	40.93 ± 0.073^{ab}	40.95 ± 0.073^{a}	40.57 ± 0.073^{b}	0.001
8	40.83 ± 0.093	41.12 ± 0.093	40.97 ± 0.093	0.093
9	41.01 ± 0.085	40.91 ± 0.085	40.85 ± 0.085	0.406
10	40.95 ± 0.118	41.09 ± 0.118	41.02 ± 0.118	0.729
11	41.17 ± 0.094^{a}	40.80 ± 0.094^{b}	41.09 ± 0.094^{ab}	0.019
12	40.73 ± 0.087^{b}	41.37 ± 0.087^{a}	41.22 ± 0.087^{ab}	0.000
13	40.68 ± 0.094^{b}	41.31 ± 0.094^{a}	41.26 ± 0.094^{ab}	0.000
14	40.72 ± 0.06^{b}	41.23 ± 0.096^{ab}	41.32 ± 0.108^{a}	0.000
15	40.85 ± 0.092^{b}	41.36 ± 0.095^{a}	41.28 ± 0.107^{ab}	0.001
16	40.71 ± 0.090^{b}	41.23 ± 0.093^{ab}	41.26 ± 0.105^{a}	0.000
Total	40.68 ± 0.032^{b}	41.13 ± 0.033^{a}	41.07 ± 0.034^{ab}	0.000

^{a,b}Means with different superscripts in the same row differ significantly (P < 0.05).

Effect of honey supplemented in drinking water on heart rate of laying chickens during the hot-dry season is presented in Table 3. It was observed that control group had a significantly lower ($p < 0.05$) HR compared to honey treatment groups in week 1. However, opposite is the case in weeks 8 – 10, 12 and 13 where HR was significantly high ($p < 0.05$) in control group than the honey treated groups. There were no significant difference in week 2 – 7, 11 and 14 – 16. The highest overall HR was recorded in 0H group (313 ± 1.70 bpm) while the overall lowest was in 20H layers (300.9 ± 1.70 bpm). The control birds had the mean highest HR in all the treatments which shows a positive impact of honey addition to laying chickens during the hot-dry season. This is in agreement with Osakwe and Igwe (2015) who also reported a lower HR in laying birds given 20 H of honey during the hot-dry season. Reduction in HR could mean that the heart is faced with less stress and is not pumping blood at a higher force as found in heat-stressed birds. The use of vitamin C has been known to decrease the amount of corticosteroids released during stress (Sahin *et al.*, 2003), making it play an important role in response to stress.

The response of the packed cell volume (PCV), heterophils - lymphocytes ratio and differential count are presented in Table 4. Lymphocytes and basophils were significantly affected ($p > 0.05$) by honey supplemented water in the laying chickens during hot-dry season. Lymphocytes count was significantly higher ($p < 0.05$) in 20 H birds (50.6 ± 0.79 %) while basophils count was significantly higher in 10 H birds (3.1 ± 0.39 %). However there was no significant ($P > 0.05$) effect of honey on PCV, heterophils, monocytes, eosinophils and H:L.

During heat stress, reduced lymphocyte count is observed (Altan *et al*., 1999). In this study, lymphocytes count increased with increasing levels of honey which could be due to the presence of foreign substance in the blood system in response to the immune system. Higher values in basophils, heterophils and H:L ratio in the 0 H

Table 3: Effect of honey supplementation on heart rate (beats per minutes) of laying chicken during hot-dry season

Week	0 H	10 H	20 H	P-value
1	255.20 ± 2.915^{b}	266.80 ± 2.915^{ab}	283.3 ± 2.915^{a}	0.000
2	285.07 ± 0.588	279.20 ± 0.588	282.40 ± 0.588	0.458
3	293.65 ± 1.343	305.56 ± 1.343	301.88 ± 1.343	0.787
4	317.87 ± 5.577	308.13 ± 5.577	322.13 ± 5.577	0.203
5	277.87 ± 5.372	288.80 ± 5.372	280.40 ± 5.372	0.331
6	323.47 ± 6.256	306.40 ± 6.256	312.53 ± 6.256	0.161
7	305.07 ± 3.970	292.53 ± 3.970	293.20 ± 3.970	0.053
8	321.73 ± 5.116^{a}	305.87 ± 5.116^{ab}	302.40 ± 5.116^{b}	0.024
9	325.60 ± 4.884^{a}	309.33 ± 4.884^{ab}	303.20 ± 4.884^{b}	0.007
10	329.07 ± 4.535^{a}	312.00 ± 4.535^{b}	311.73 ± 4.535^{ab}	0.013
11	324.80 ± 5.628	312.53 ± 5.628	305.07 ± 5.628	0.054
12	328.27 ± 4.618^{a}	313.07 ± 4.618^{ab}	309.87 ± 4.618^{b}	0.017
13	322.40 ± 5.324^{a}	311.20 ± 5.324^{ab}	300.00 ± 5.324^{b}	0.018
14	338.40 ± 4.611	304.00 ± 4.611	299.33 ± 5.155	0.000
15	322.93 ± 6.369	308.00 ± 6.593	305.09 ± 7.438	0.141
16	319.20 ± 4.616	306.86 ± 4.779	304.00 ± 5.391	0.074
Average	313.13 ± 1.660^{a}	301.60 ± 1.667^{b}	300.87 ± 1.702^{ab}	0.000

^{a,b}Means with different superscripts in the same row differ significantly ($P < 0.05$).

Table 4: Haematological indices of Layers fed different levels of Honey

Parameter	0 H	10 H	20 H	P-value
PCV	24.0 ± 1.74	24.0 ± 1.74	23.8 ± 1.74	0.993
Lymphocytes	41.3 ± 0.79^{b}	49.5 ± 0.79^{ab}	50.8 ± 0.79^{a}	0.017
Heterophils	38.5 ± 1.12	32.5 ± 1.12	30.8 ± 1.12	0.158
Basophils	8.3 ± 0.39^{a}	3.3 ± 0.39^{b}	5.0 ± 0.39^{ab}	0.017
Monocytes	10.0 ± 0.72	12.5 ± 0.72	9.5 ± 0.72	0.463
Eosinophils	2.5 ± 0.43	2.5 ± 0.43	4.5 ± 0.43	0.405
H:L	0.9 ± 0.09	0.7 ± 0.09	0.6 ± 0.09	0.058

^{a,b}Means with different superscripts in the same row differ significantly ($P < 0.05$), PCV = Packed Cell Volume, H:L = Heterophil-Lymphocyte ratio.

layers showed that the hens were heat stressed (Maxwell *et al*., 1992). 10 H layers had the lowest basophil and H:L ratios which implies that honey administered to laying chicken during hot-dry season helped reduce the effect of heat stress. This could be as a result of the phytochemicals present in honey. A slight difference in eosinophil count was observed in all treatments. Flavonoids present in honey helps increase immunity of body of laying hens by decreasing their stress (Frankic *et al*., 2009).

CONCLUSION

The use of 20 ml honey per 1 L of water reduced heart rate of laying chicken in the hot – dry season. However, honey supplemented in water did not improve the rectal temperature of the layers. Basophils count was reduced in honey treatment layers while lymphocytes count was increased. Further research should be carried out on the use of smaller quantities of honey on pullets.

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