

Full Length Research Paper

Physiological performance of heat stressed growing rabbits fed diets supplemented with vitamin anti-oxidants and bicarbonate buffers

Anoh K. U.* , Ayuba D., Ozung P. O. and Udayi M. A.

Department of Animal Science, University of Calabar, Calabar Nigeria.

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The aim of the study was to evaluate the physiological performance of heat stressed growing rabbits fed diets supplemented with vitamin anti-oxidants and bicarbonate buffers. A total of thirty (30) growing rabbits (New Zealand White crosses) of two months old were used in this study. There were six (6) rabbits per treatment in a Completely Randomized Design (CRD). The treatment groups consisted of: control, sodium bicarbonate (NaHCO_3) and potassium bicarbonate, (KHNO_3) solution respectively, Vitamin C, and baobab fruit pulp meal (BFPM) as supplements, respectively. The experiment lasted for 9 weeks. Five milliliters of blood were collected from five rabbits (through the ear vein) chosen randomly from each group of rabbits, respectively at the beginning and the end of the experiment for serum metabolite and thyroxine hormone evaluation. Physiological performance of the rabbits was also evaluated. It was found that environmental conditions were stressful to the animals; Vitamins (Vit C and BFPM) significantly ($P<0.05$) reduced rectal temperature, heart rate and triglycerides compared to other treatments. The buffers recorded significantly ($P<0.05$) high feed intake, calcium and thyroxine. It was concluded that ameliorating heat stress with the antioxidants were helpful to improve the performance of rabbits and was recommended to be included in rabbit diets during the hot period.

Key words: Antioxidants, buffers, heat stress, thyroxine.

INTRODUCTION

Rabbits have been identified as a micro livestock with high economic potentials that can be used to bridge the gap for dietary protein intake in Nigeria. Rabbit meat poses a lot of health benefits by supplying micro nutrients which are deficient in leguminous grains and it is also low

in cholesterol. Rabbit as a micro livestock can play a vital role in solving the world problem of deficiency of animal proteins (Daader et al., 2016) because of their high-quality protein, short generation interval, prolificacy and fast growth rate (Lebas et al., 1986). Improvement and

*Corresponding author. E-mail: Kevin2us.man@gmail.com.

increasing rabbit population have a significant role in the mitigation of poverty in the developing countries, where it is raised. Rabbit enterprise has also a main role in employment of rural communities' population.

Heat stress has been a major challenge in tropical and subtropical countries on rabbit production leading to impairment of both productive and reproductive performance (Marai et al., 2004, 2001). During stress, the endocrine system suffers (Chauhan et al., 2014). Adrenal corticosteroids are secreted during heat stress. High level glucocorticoids accelerate the metabolic rates and increase free radicals especially reactive oxygen species (ROS) (Ross et al., 1985; Sivakumar et al., 2010; Chauhan et al., 2014). Although low levels of ROS are essential for many biochemical processes, their accumulation due to over-production or a decreased antioxidant defense, leads to damage of biological macromolecules and disruption of normal cell metabolism (Spurlock and Savage, 1993). During heat stress, the few antioxidants in the body are depleted and induce oxidative stress (Abou-Ashour et al., 2004).

Vitamin C (ascorbic acid) and E are vitamin antioxidants that have been widely used to alleviate heat stress in rabbits and poultry (Yassein, 2010; Arafa Mervat et al., 2012). Vitamin C is prominent in the defense against superoxide ions, singlet oxygen and other free radicals. It also neutralizes RNA. Ascorbic acid has the potential of dissolving in blood and cytosol and can act quickly before cell damage occurs. Plants and their parts could serve as a phytobiotics and antioxidants to the livestock (Dhama et al., 2015; Valenzuela-Grijalva et al., 2017) because of the presence of vitamin C and other phytochemicals in them. Some phytochemicals in plants improve antioxidant, anti-microbial, feed flavour and palatability which could result in increased feed intake and performance in animals (Valenzuela-Grijalva et al., 2017). These tropical plants are available because of their rapid growth which is enhanced by the prevailing and environmental factors. Baobab has been described by Williams (2002) and Phyto Trade Africa (2009). The fruit pulp was reported to contain high amounts of vitamin C (Sena, 1998), it was reported to increase feed intake, weight gain and was effective in alleviating heat stress in rabbits (Anoh, 2017). NaHCO_3 was found to improve oxidative stress and heat tolerance by immune-modulation. Sodium bicarbonate in feed or water was reported to improve growth performance (Ahmad et al., 2005; Khattak et al., 2012; Peng et al., 2013), egg quality (Kaya et al., 2004; Jiang et al., 2015) and improve blood profile (Kurtoglu et al., 2007) in poultry birds and rabbits that were affected by heat stress. The chemical is cheap, easily available and easy to handle, therefore, can be safely used to ameliorate the adverse effects of heat stress.

This study was designed to evaluate effect of vitamin anti-oxidants and bicarbonate buffers on physiological

performance and thyroxine secretion of heat stressed growing rabbits.

MATERIALS AND METHODS

Experimental site

This study was carried out at the Rabbit Unit of the National Animal Production Research Institute (NAPRI) Shika, Zaria. Shika lies between $11^{\circ} 12' 42''$ N and $7^{\circ} 33' 14''$ E at an altitude of 691 m above sea level (Ovimaps, 2014). Zaria has an average rainfall of 1100 mm which starts from late April and early May to mid-October and an average temperature of 37°C and average relative humidity of 75%.

Preparation of buffer

Potassium bicarbonate, sodium bicarbonate and carbonate anhydrous salts were purchased from a laboratory equipment and chemicals vendor in Samaru-Zaria Nigeria. Distilled water was prepared in the Multiuser Laboratory of the Department of Chemistry, Ahmadu Bello University, Zaria. The buffer solution was prepared according to the methods of Chandra (2006) at a pH of 7.5 in the Department of Biochemistry, Ahmadu Bello University, Zaria.

Experimental animals, diets and design

A total of thirty (30) growing rabbits (New Zealand White crosses) of two months old were used in this study. There were six (6) rabbits per treatment in a Completely Randomized Design (CRD). The treatment groups consisted of: control, sodium bicarbonate (NaHCO_3) and potassium bicarbonate, (KHNO_3) solution, respectively, Vitamin C, and baobab fruit pulp meal (BFPM) as supplements, respectively. The basal diet composition (kg) was as follows: maize 30, groundnut haulm 20, groundnut cake 10, soybean meal 15, rice bran 15, bone meal 9.2, common salt 0.35, mineral-vitamin premix 0.25, dl-methionine 0.1 and lysine 0.1. The basal diet was formulated to meet the nutrient requirements of growing rabbits according to the recommendations of NRC (1994). The calculated analysis of the nutrient composition of the basal diet is shown in Table 1. The buffer solution was offered *ad libitum* but changed daily in the morning. All recommended managerial practices were duly observed and the study lasted for 9 weeks.

Housing

The animals were housed in perforated metallic hutches measuring $75 \times 75 \times 75$ cm and raised 80 cm from the floor level in a naturally ventilated building. The hutches were thoroughly washed and disinfected with a locally made disinfectant and allowed to dry for one week before the animals were brought. Feed and watering troughs which were made of burnt clay were provided in each hutch. The rabbits were placed individually in clearly labeled cells.

Meteorological data of rabbit microclimate

The microclimate (ambient temperature and relative humidity values) within the rabbit house was recorded twice daily at 08:00 and 15.00 h during the study period using a digital thermometer

Table 1. Calculated composition of the experimental diets.

Nutrients	Composition
Metabolizable energy (kcal/kg)	2200
Crude protein (%)	18
Crude fiber (%)	10

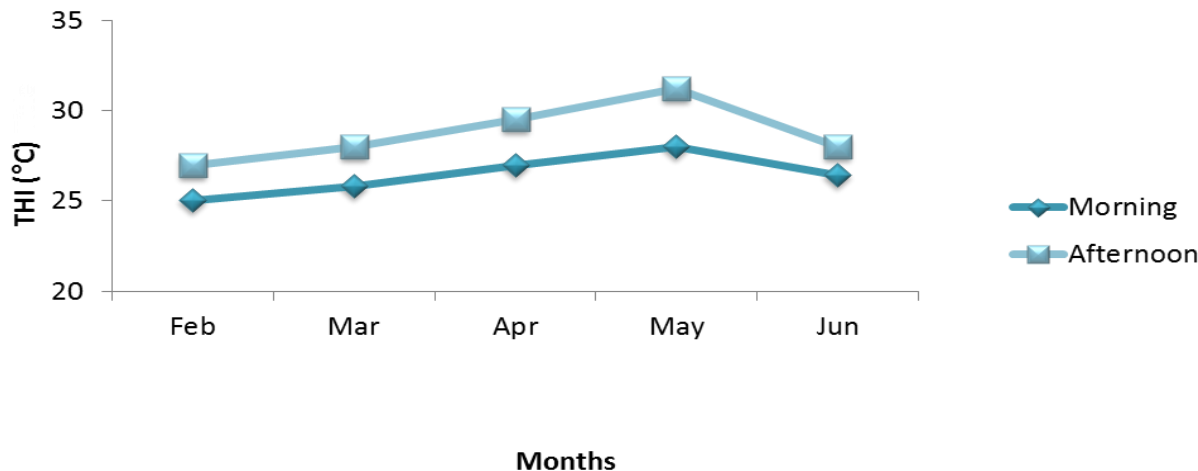


Figure 1. Monthly temperature humidity index of the pen house.

(Cocet, Shenzhen-Guangdong, China). The data collected was used to compute the temperature-humidity index (THI), an indicator of the thermal comfort level of the rabbits. The THI was calculated using the modified formula for the rabbit by Marai et al. (2001) as follows:

$$THI = t - [(0.31 - 0.31 \times RH) (t - 14.4)]$$

where RH = relative humidity /100 and t = ambient temperature.

The values of THI obtained were compared to that classified for tropical regions as follows:

- 1) <27.8 = Absence of heat stress,
- 2) 27.8 - 28.9 = Moderate heat stress,
- 3) 28.9 – 30 = Severe heat stress,
- and 4) above 30 = Very severe heat stress.

Physiological performance evaluation

Measurements of rectal temperature (RT) and heart rate (HR) were taken at 14.00 to 15.00 h of the day. Rectal temperature was measured with a digital thermometer and HR was measured by counting the heartbeat of each rabbit representing their treatment for 1 min with the help of a stethoscope. Weight gain and feed intake was determined weekly.

Serum evaluation

Blood sampling was done every two weeks at 10.00 h. Four rabbits were randomly selected from each treatment group and 5 ml of blood was collected from their ear veins into sample bottles without

anticoagulants. The blood sample was allowed to clot and the serum was harvested after centrifuging the samples at 3000 rounds/min for 15 min. The serum harvested was stored at -10°C until when analyzed. Serum thyroxine concentrations were determined by using commercially available ELISA Kits (Diagnostic Procedure Corp., Los Angeles, CA, USA) according to the manufacturer’s instructions. Serum glucose, total protein, albumin, and cholesterol concentration were evaluated using an auto-analyzer and Chemical Commercial Kits from Stanbio Laboratory Inc. San Antonio, Texas, USA, according to the manufacturer’s instruction. The detection range of the T₄ was 26 to 58.4 g/mg.

Statistical analysis

Data obtained from the study were subjected to analysis of variance using the general linear model procedure of SAS (2002). Significant differences among treatment means were separated using the pairwise difference (Pdiff) in the SAS package.

RESULTS

Monthly temperature-humidity index (THI)

The monthly temperature-humidity index (THI) inside the rabbitry during the experimental period is as shown in Figure 1. THI in the mornings averaged 26.44°C while the afternoon THI averaged 28.74°C. This graph also shows that the THI values kept increasing from the month of

Table 2. Effects of vitamin anti-oxidants and bicarbonate buffers on physiological performance of growing rabbits treatments.

Parameter	Control	KHCO ₃	NaHCO ₃	Vit. C	BFPM	SEM
Rectal temperature (°C)	37.68 ^a	36.56 ^{bc}	37.27 ^a	35.87 ^c	35.73 ^c	0.31
Heart rate (beats/min)	141.03 ^b	143.36 ^a	143.19 ^a	141.35 ^b	141.29 ^b	0.53
Feed intake (g/day)	30.76 ^b	30.93 ^b	42.39 ^a	37.59 ^a	36.77 ^a	1.88
Weight gain (g/day)	13.96	16.99	14.58	13.37	15.28	1.82

Means within rows with different superscripts are significantly different: $p < 0.05$, SEM= Standard Error of Mean, Vit. C = Vitamin C, BFPM = Baobab fruit pulp meal

Table 3. Effects of vitamin anti-oxidants and bicarbonate buffers on serum metabolites of growing rabbits treatments.

Parameter	Control	KHCO ₃	NaHCO ₃	Vit. C	BFPM	SEM
Glucose (mg/dl)	3.50	3.30	3.60	4.80	5.00	0.12
Total Protein (mg/dl)	66.67	65.67	72.00	69.67	72.67	0.38
Albumin (mg/dl)	34.67	36.00	37.67	37.33	38.67	1.38
Cholesterol (mg/dl)	1.33	1.30	1.33	1.43	1.43	0.04
Triglyceride (mg/dl)	0.97 ^b	1.00 ^{ab}	1.00 ^{ab}	1.30 ^{ab}	1.40 ^a	0.08
Calcium (mg/dl)	2.39 ^{ab}	2.31 ^{ab}	2.30 ^{ab}	2.33 ^a	2.26 ^b	0.01
Phosphorous (mg/dl)	1.08	1.13	1.31	1.16	1.09	0.01

Means within rows with different superscripts are significantly different: $p < 0.05$, SEM= Standard Error of Mean, Vit. C = Vitamin C, BFPM = Baobab fruit pulp meal.

February with a peak in May. There was a decline in THI in the month of June.

Physiological evaluation

Vitamins (Vit C and BFPM) (Table 2) significantly ($p < 0.05$) reduced rectal temperature and heart rate compared to other treatments. The range values were 37.68 (control) to 35.73 (BFPM). Feed intake significantly ($p < 0.05$) increased in the treatments with NaHCO₃, Vitamin C, and BFPM (42.39, 37.59, and 36.77), respectively compared to KHCO₃ (30.93) and the control (30.76).

Serum metabolite evaluation

There was a significant ($p < 0.05$) increase in the values of triglycerides (Table 3) for the Vitamins (1.30 and 1.40) compared to the buffers (1.00) and the control (0.9). Vitamin C recorded significantly ($p < 0.05$) higher calcium compared to BFPM which was the lowest among the treatments.

Thyroxine secretion evaluation

The result in Figure 2, shows that Initial thyroxine levels

were generally low compared to final thyroxine secretions. The trend in the values of the final thyroxine secretions was a reflection of the trend in the thyroxine of the initial secretions. The buffers recorded significantly ($P < 0.05$) higher thyroxine levels (67.12 and 66.92) compared to treatments with vitamins (65.45 and 66.10). Going by the difference in initial vs final thyroxine secretion (3.46 vs 3.60) of NaHCO₃ and BFPM, respectively, it was observed that the difference in the increase was higher for BFPM treated rabbits than NaHCO₃ treatment.

DISCUSSION

The THI value of 27°C (February) indicated that the month of February had absence of heat stress in the rabbit house, while the THI values of 28°C (March), 29.5°C (April), 31.2°C (May) and 28°C (June) are indications that the rabbit house was moderately thermally stressful, severely thermally stressful, and very severely thermally stressful (Marai et al., 2001) in these months. The averaged THI 28.74°C during the experimental period indicated that the rabbit house was thermally stressful and may have had adverse effects on the rabbits (Marai et al., 2001). Overall data obtained indicated that THI in the afternoon was higher by 1.24% than THI in the morning.

The increase in feed intake in KHCO₃ treatment agrees

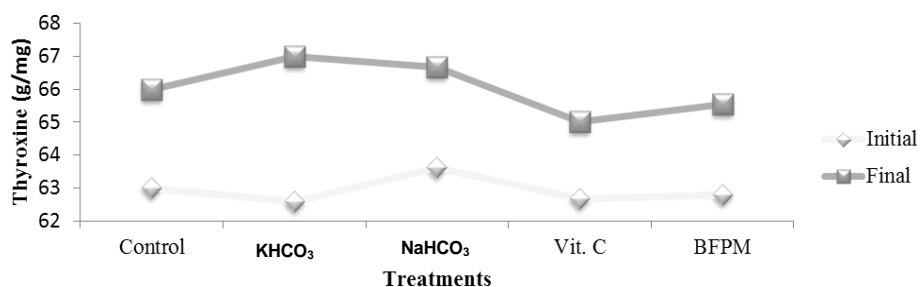


Figure 2. Effects of vitamin anti-oxidants and bicarbonate buffers on thyroxine levels in growing rabbits.

with the findings of Yassein et al. (2011) who reported that NaHCO₃ and KHCO₃ can serve as appetizer supplement to rabbits diets which may stimulate the appetite, increase fiber digestibility and improve feed efficiency (Abdel-Samee et al., 2003). Sodium bicarbonate in feed or water has shown potential benefits on production performance (Ahmad et al., 2005; Khattak et al., 2012; Peng et al., 2013), egg characteristics (Kaya et al., 2004; Jiang et al., 2015) and blood profile (Kurtoglu et al., 2007) in poultry birds and rabbits exposed to heat stress.

Marai and Habeeb (1994) found that 1.25 or 2.5% NaHCO₃ improved growth performance, rectal temperature, respiration rate and blood components due to correcting acid-base balance disturbances, such as under stress conditions. Vitamin C has been reported to be effective in the growth performance of rabbits especially during heat stress (Rao and Sharma, 2001). Antioxidant vitamins were effective to alleviate heat load in rabbits (In-Surk et al., 2014; Prabsattroo et al., 2012). The administration of ascorbic acid (Mckeeand and Harrison, 2013), glutathione supplementation (Sahin et al., 2003), during exposure to high environmental temperatures reduces the body temperature in chickens. In heat-stressed sheep, selenium injection decreased rectal temperature and body weight loss (Alhidary et al., 2012). The presence of the phytochemical compounds in plants, vitamins may facilitate the ability of animals to maintain their body homeostasis including body temperature by provoking endogenous cellular defense mechanisms to cope with oxidative stress and inflammation induced by heat stress (Akbarian et al., 2016).

The values in final serum metabolites in this experiment followed the trend of the rabbit's growth performance records (feed intake, weight gain and final body weight) in this study. For instance, BFPM recorded a significantly high serum triglyceride which was similar to the treatments with vitamin C and bi-carbonate buffer compared to the control. It should be recalled that these treatments were the ones that showed a better feed

intake and weight gain compared to the control. Triglycerides are an important component of the body's adipose tissue. Ambient temperature-induced heat stress was shown to reduce fat oxidation in different species; rodents (Sanders et al., 2009), pigs (Pearce et al., 2011) and dairy cows (Shwartz et al., 2009). Moreover, heat stress down-regulates lipolytic enzyme activities, as seen in chickens and swine (Geraert et al., 1996). Lebas et al. (1986), Chiericato et al. (1996) and Marai et al. (2001) reported a reduction in live body weight and daily body gain weight due to heat-stress conditions and were attributed to the negative effects of heat-stress on appetite and consequent decrease in feed consumption. Low feed intake might have affected the low serum metabolite noticed in the control group. The blunted lipolytic activity of the adipose tissue seems to be an adaptation form to limit heat generation in heat-stressed animals.

The marked differences in initial and final thyroxine levels can be attributed to changes in age and body metabolism of the rabbits. It should be known that body metabolism increases with age; T₄ have been reported to increase with increasing age in chicks (Leenstra et al., 1991). In the present study the buffers recorded a high thyroxine secretion; the buffer might have triggered the activity of the thyroid gland to increase thyroxine secretion. On the reduction in T₄ at the final stage by the treatments with vitamin C and BFPM meal diets agrees with the fact that thyroid the hormone increases the quantities of many bodily enzymes and because vitamins are essential parts of some of the enzymes or coenzymes, thyroid hormone causes increased need for vitamins (Guyton and Hall, 2006). Therefore, a relative vitamin deficiency can occur when excess thyroid hormone is secreted, this will lead to a consequential depletion and the need for more vitamins to maintain homeostasis and will, in turn, reduce thyroxine secretion, unless at the same time increased quantities of vitamins are made available. Serum concentrations of T₄ increased by increasing dietary Vitamin C or Vitamin E levels of heat-stressed Japanese quails (Sahin et al.,

2002) and rabbits (Daader et al., 2018). Thyroid hormones are the key hormones in the regulation of metabolism and adaptation of animals to stress (Brecchia et al., 2010).

Conclusion

Ameliorating heat stress with the antioxidants was helpful to improve the performance of rabbits. Vitamin antioxidants performed better than the bicarbonate buffers and were recommended to be included in rabbit diets during the hot period.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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