Current Journal of Applied Science and Technology

22(2): 1-5, 2017; Article no.CJAST.34343
Previously known as British Journal of Applied Science & Technology
ISSN: 2231-0843, NLM ID: 101664541

Enzymatic Response to Antioxidants and Seasonal Stress

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AC, Anubha Baruah, BCS, JG, Arundhati Bora, DJD, RKB and DK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AC, SN and YV managed the analyses of the study. Author DP managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2017/34343
Editor(s):
(1) Ahmed Mohamed El-Waziry, King Saud University, College of Food and Agriculture Sciences, Kingdom of Saudi Arabia.
Reviewers:
(1) Seema Akbar, Kashmir University Campus, India.
(2) Priscila de Oliveira Moraes, Universidade Federal do Rio Grande do Sul, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/19979

Original Research Article

Received 24th May 2017
Accepted 18th June 2017
Published 10th July 2017

ABSTRACT

The present experiment was conducted to study the activity of Superoxide dismutase (SOD) enzyme in the crossbred pigs (Hampshire × Local) under the agro-climatic condition of Assam. The experiment included a total of 36 numbers of crossbred weaned female pigs. Eighteen (18) animals

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were subjected to treatment separately during summer and winter. The selected animals were divided into three groups with six pigs in each group consisting of the control group (Treatment 1), one group was fed melatonin @3 mg/animal (Treatment 2) and the other group was fed Vitamin E @100 mg (Treatment 3) for both the seasons. The animals were maintained at AICRP on Pig, College of Veterinary Science, AAU, Khanapara, Guwahati-22.
Temperature-Humidity Index was calculated out from the data of ambient temperature and relative humidity by using standard formula. About 5 ml of blood was collected from each experimental animal aseptically at 15 days interval for the whole experimental period. The enzyme superoxide dismutase (SOD) was estimated by using SOD assay kit manufactured by Cayman Chemical Company, USA as per manufactures protocol.
The Temperature Humidity Index (THI) during the study period was indicative of thermal stress to the experimental animals in the summer (82.01±0.50) as compared to winter season (63.16±0.30). The serum SOD activity was found to differ significantly ($P<0.01$) higher between treatment and between season and also between treatment and season.

Keywords: Antioxidants; enzymatic; stress; season.

1. INTRODUCTION

Heat is generated in every living cell of an animal as it metabolizes nutrients. As environmental temperature increases, the heat generated within the body of the animal is increasingly more difficult to dissipate to the surroundings. When heat production exceeds heat dissipation, body temperature rises. Swine are particularly susceptible to heat stress because they possess little to no functional sweat glands [1]. In addition, pigs maintain more subcutaneous fat compared to other species and this prevents effective heat dissipation [2]. Heat stress is one of the wide varieties of factors which cause oxidative stress *in-vivo*. Reactive oxygen species (ROS), the major culprits for causing oxidative stress, are constantly generated *in vivo* as an integral part of metabolism. ROS may cause oxidative stress when their level exceeds the threshold value. They trigger progressive destruction of polyunsaturated fatty acids (PUFA), ultimately leading to membrane destruction. Enzymatic activity serves as a tool for determining the level of heat stress in animals.

2. MATERIALS AND METHODS

2.1 Place of Work

The present study was carried out at the Department of Veterinary Physiology and AICRP on Pig, College of Veterinary Science, Khanapara, Guwahati and National Research Centre on Pig, Rani.

2.2 Period of Work

The experimental study was carried out during two different seasons: Summer (June, July & August, 2014) and winter (December, 2013 & January and February 2014).

2.3 Experimental Design

The present experiment included 36 nos. of weaned, healthy and uniform sized crossbred (Hampshire X Assam local) female pigs. Eighteen (18) animals were subjected to treatment separately during summer and winter. The selected animals were divided into three groups with six pigs in each group consisting of the control group (Treatment 1), animals of one group was fed melatonin (Meloset) @3 mg/animal (Treatment 2) and the other group was fed Vitamin E (Evion) @100 mg (Treatment 3) for both the seasons. The animals were fed as per standard feeding practices of the farm. For identification of the animals, numbers were imprinted by trimming the body hairs.

2.4 Temperature-Humidity Index (THI)

Temperature-Humidity Index was calculated out from the data of ambient temperature and relative humidity [3]. The dry bulb temperature and relative humidity were recorded daily from June to August, 2014 and December 2013 to February 2015 from the Automatic Weather Station (AWS) installed in the College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, where the experimental animals were reared. Temperature-Humidity Index was calculated for the entire period using the following formula:

$$THI = (0.8 \times Tdb) + [(RH/100) \times (Tdb - 14.4)] + 46.4$$

2.5 Blood Collection

About 5 ml of blood was collected from each experimental animal aseptically at 15 days interval for the whole experimental period.

2.6 Estimation of Enzyme Activity

The enzyme superoxide dismutase (SOD) was estimated by using SOD assay kit manufactured by Cayman Chemical Company, USA as per manufactures protocol. The estimation used 96 wells ELISA plates namely Dynamica, Halo MPR 96 visible Microplate Readers (Australia). The results obtained were expressed in U/ml.

3. RESULTS AND DISCUSSION

The Temperature Humidity Index (THI) during the study period was 82.01±0.50 in summer and 63.16±0.30 winter season (Tables 1 and 2). It was significantly different (P<0.01) between seasons.

Table 1. Temperature humidity index (THI) (Mean± SE) during summer and winter season

Season	THI
	Mean± SE
Summer	82.01±0.50
Winter	63.16±0.30

The present findings are in close relation to those reported earlier. They reported that THI level beyond 72 was indicative of mild heat stress, THI 75 to 78 denoted stressful condition and that beyond 78 could indicate severe stress due to heat and humidity [4]. Temperature and humidity conditions also affected livestock production in Central Argentina [5]. Davis and Mader, reported that the Temperature-Humidity Index (THI) is a suitable climatic marker to correlate climatic stress on physiology and productivity of animals and also a reliable tool for effective management of livestock under different climatic condition [6].

The mean SOD values in the three treatment groups during summer and winter are presented in Tables 3 and 4 respectively. The mean SOD values during summer was found to be 1.87±0.10 in treatment group 1, 1.80±4.67E-03 in treatment group 2 and 1.84±0.01 in treatment group 3. On the other hand the mean SOD value during winter in the three treatment groups was found to be 1.18±4.69E-03 in treatment group 1, 1.18±4.00E-03 in treatment group 2 and 1.19±3.95E-03 in treatment group 3.Statistical analysis revealed significant difference (P<0.01) in the mean SOD values between treatment and between season. There was also significant difference (P<0.01) between treatment and season.

In the present study mean SOD values during summer was found 1.87±0.10 in treatment group 1, 1.80±4.67E-03 in treatment group 2 and 1.84±0.01 in treatment group 3 with an aggregate of 1.78±0.01 whereas in winter it was 1.18±4.69E-03 in treatment group 1, 1.18±4.00E-03 in treatment group 2 and 1.19±3.95E-03 in treatment group 3 with an aggregate of 1.18±2.45E-03^{b}.

Table 2. ANOVA for temperature humidity index (THI) during summer and winter season

Parameters	Source of variation	Sum of squares	Df	Mean square	F	P Value
THI	Between season	532.984	1	532.984	1058.593	<0.001**
	Within season	2.014	4	0.503		
	Total	534.998	5			

***P(<0.001)*

Table 3. Serum SOD (U/ml, Mean± SE) concentration in the different treatment groups DURING summer and winter season

Treatment	Season		Aggregate
	Summer (Mean±SE)	Winter (Mean±SE)	
1	1.87±0.01^{a}	1.18±4.69E-03^{d}	1.53±0.04^{a}
2	1.80±4.67E-03^{b}	1.18±4.00E-03^{d}	1.49±0.04^{b}
3	1.84±0.01^{c}	1.19±3.95E-03^{d}	1.51±0.03^{c}
Aggregate	1.83±0.01^{a}	1.18±2.45E-03^{b}	1.51±0.02

E stands for 10 and figure after E is power of 10, Values having same superscript do not differ significantly

Table 4. ANOVA for serum sod concentration in the different treatment groups during summer and winter season

Source	Sum of squares	Df	Mean square	F	P value
Replication	0.042	5	0.008	4.963	<0.001
Treatment	0.414	2	0.207	122.309	<0.001**
Error (Treatment)	0.061	10	0.006		
Season	19.147	1	19.147	11325.117	<0.001**
Treatment * Season	0.487	2	0.243	144.000	<0.001**
Error (Season)	0.330	195	0.002		
Total	20.480	215			

The present findings are in close proximity with the findings earlier reported by. The mean activity of serum SOD during summer in pre and post sunshine Beetal goats as 1.81±0.01 and 1.72±0.01 U/ml respectively. The corresponding activity in the melatonin fed group was 1.83±0.01 and 1.77±0.01 /ml respectively. On the other hand during winter season the mean activity of serum SOD in pre and post sunshine exposed Beetal goats was 1.15±0.01 and 1.17±0.01 /ml, respectively. The corresponding values in the melatonin fed group was 1.17± 0.01 and 1.20±0.01 U/ml, respectively [7].

There is excessive production of reactive oxygen species (ROS) such as superoxide anion, hydroxyl ion and hydrogen peroxide during thermal. They are continuously produced in the course of normal aerobic metabolism. If these free radicals are not eliminated they can damage the healthy cells. This may result in disturbed physiology and altered biochemical profile of the animal [8]. Superoxide dismutase, catalase and glutathione peroxidase are the major defense in the detoxification of superoxide anion and hydrogen peroxide [9,10]. Superoxide dismutase along with catalase and glutathione peroxidise scavenges both intracellular and extracellular superoxide radicals and prevents lipid peroxidation [11]. Superoxide dismutase that catalyzes dismutation of superoxide becomes important in the defense mechanisms against oxidative stress [12]. The SOD activity in summer was lowest in the melatonin supplemented group i.e., treatment 2.Researchers suggested that decrease SOD activity is due to consumption of SOD to overcome the oxidative stress [13]. This might also be due to the antioxidant effects of melatonin that scavenges the free radicals generated during heat stress [14]. In winter little variation in the SOD activity was witnessed and they did not differ significantly which is suggestive of the comfortable environment the animals were in.

4. CONCLUSION

The experiment conducted reveals that THI is a very important biomarker in assessing the seasonal stress which can cause significant variation in the enzymatic profile of animals. It also dictates the protective action of antioxidants against cell damage.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Curtis SE. Environmental management in animal agriculture. Ames; Iowa State University Press; 1983.
2. Mount LE. Adaptation to thermal environment: Man and his productive animals. Edward Arnold Limited, Thomson Litho Ltd, East Kilbride, Scotland; 1979.
3. Mader TL, Kreikemeier WM. Effects of growth promoting agent and seasons on blood metabolites and body temperature in heifers. J. Anim. Sci. 2006;84(4):1030-1037.
4. Kadzere CT, Murphy MR, Silanikove N, Maltz E. Heat stress in lactating cows: A review. Livestock Prod. Sci. 2002;77:59-91.
5. Antonio C, Andres CR. Temperature and humidity conditions affecting livestock production in Central Argentina. International Journal of Biometeorology. 2003;48(1):6-9.
6. Davis MS, Mader TL. Accounting wind speed and solar radiation in temperature humidity index. American Society of Anim. Sci. 2002;42:137-139.
7. Hasin D. Thermal stress physiology and effect of melatonin on goats with special reference to hsp70 under agro-climatic

conditions of Assam. PhD thesis, Assam Agricultural University, Khanapara, Guwahati-22; 2015.
8. Bernabucchi U, Ronchi B, Lacetera N, Nardone A. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. J. Dairy Sci. 2002;85:2173-2179.
9. McCord JM, Fridovich I. Superoxide dismutase: An enzymatic function for erythrocuprein (Hemocuprein). J. Biol. Chem. 1969;244:6049-6055.
10. Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiol. Rev. 1979;59:527-605.
11. Agarwal A, Prabhakaran SA. Mechanism, measurement and prevention of oxidative stress in male reproductive physiology. Indian J. Exp. Biol. 2005;43:963-97.
12. Halliwell B, Chirico S. Lipid peroxidation: Its mechanism, measurement and significance. Am. J. Clin. Nutr. 1993;57: 715S-724S.
13. Al-Badwy MA, Mohammed H. E. Abudabes, Alhaidry AMA, Al-Hassaa MJ. The effects of transportation on and oxidative biomarkers, rectal and skin temperatures in Aardi goats. Indian J. Anim. Res. 2013;47:392-396.
14. Ahmed HH, Essawy GS, Salem HA, Abdel Daim MA. Melatonin has a strong antioxidant activity and improves liver and kidney functions in broiler chicks. Egypt. J. Basic and Appl. Physiol. 2005;4(1):77-92.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/19979