

1 ENZYMATIC RESPONSE TO ANTIOXIDANTS AND SEASONAL STRESS

2 ABSTRACT

3 The present experiment was conducted to study the activity of Superoxide dismutase
4 (SOD) enzyme in the crossbred pigs (Hampshire × Local) under the agroclimatic condition
5 of Assam. The experiment included a total of 36 numbers of crossbred weaned female pigs.
6 Eighteen (18) animals were subjected to treatment separately during summer and winter. The
7 selected animals were divided into three groups with six pigs in each group consisting of the
8 control group (Treatment 1), one group was fed melatonin @3 mg/animal (Treatment 2) and
9 the other group was fed Vitamin E @100 mg (Treatment 3) for both the seasons. The animals
10 were maintained at AICRP on Pig, College of Veterinary Science, AAU, Khanapara,
11 Guwahati-22.

12 Temperature-Humidity Index was calculated out from the data of ambient
13 temperature and relative humidity by using standard formula. About 5 ml of blood was
14 collected from each experimental animal aseptically at 15 days interval for the whole
15 experimental period. The enzyme superoxide dismutase (SOD) was estimated by using SOD
16 assay kit manufactured by Cayman Chemical Company, USA as per manufactures protocol.

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18 The Temperature Humidity Index (THI) during the study period was indicative of
19 thermal stress to the experimental animals in the summer as compared to winter season. The
20 serum SOD activity was found to differ significantly ($P<0.01$) higher between treatment and
21 between season and also between treatment and season.
22

23 INTRODUCTION

24 Heat is generated in every living cell of an animal as it metabolizes nutrients. As
25 environmental temperature increases, the heat generated within the body of the animal is
26 increasingly more difficult to dissipate to the surroundings. When heat production exceeds
27 heat dissipation, body temperature rises. Swine are particularly susceptible to heat stress
28 because they possess little to no functional sweat glands (Curtis, 1983). In addition, pigs
29 maintain more subcutaneous fat compared to other species and this prevents effective heat
30 dissipation (Mount et al., 1979). Heat stress is one of the wide varieties of factors which
31 cause oxidative stress *in-vivo*. Reactive oxygen species (ROS), the major culprits for causing
32 oxidative stress, are constantly generated *in vivo* as an integral part of metabolism. ROS may
33 cause oxidative stress when their level exceeds the threshold value. They trigger progressive
34 destruction of polyunsaturated fatty acids (PUFA), ultimately leading to membrane

35 destruction. Enzymatic activity serves as a tool for determining the level of heat stress in
36 animals.

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MATERIALS AND METHODS

39 PLACE OF WORK

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

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44 PERIOD OF WORK

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

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48 EXPERIMENTAL DESIGN

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

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59 TEMPERATURE-HUMIDITY INDEX (THI)

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61 Temperature-Humidity Index was calculated out from the data of ambient temperature
62 and relative humidity by using the formula of Mader *et al.* (2006). The dry bulb temperature
63 and relative humidity were recorded daily from June to August, 2014 and December 2013 to
64 February 2015 from the Automatic Weather Station (AWS) installed in the College of
65 Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, where the
66 experimental animals were reared. Temperature-Humidity Index was calculated for the entire
67 period using the following formula:

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$$69 \quad \text{THI} = (0.8 \times \text{Tdb}) + [(\text{RH}/100) \times (\text{Tdb} - 14.4)] + 46.4$$

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BLOOD COLLECTION

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

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.

RESULTS AND DISCUSSION

The mean SOD values in the three treatment groups during summer and winter are presented in Table 1 and 2 respectively. The mean SOD values during summer was found to be 1.87 ± 0.10 in treatment group 1, $1.80 \pm 4.67 \times 10^{-3}$ 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 $1.18 \pm 4.69 \times 10^{-3}$ in treatment group 1, $1.18 \pm 4.00 \times 10^{-3}$ in treatment group 2 and $1.19 \pm 3.95 \times 10^{-3}$ 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.

TABLE 1. SERUM SOD (U/ml, MEAN $\pm$ SE) CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

TREATMENT	SEASON		AGGREGATE
	SUMMER(MEAN $\pm$ SE)	WINTER(MEAN $\pm$ SE)	
1	1.87 ± 0.01^a	$1.18 \pm 4.69 \times 10^{-3}^d$	1.53 ± 0.04^a
2	$1.80 \pm 4.67 \times 10^{-3}^b$	$1.18 \pm 4.00 \times 10^{-3}^d$	1.49 ± 0.04^b
3	1.84 ± 0.01^c	$1.19 \pm 3.95 \times 10^{-3}^d$	1.51 ± 0.03^c
AGGREGATE	1.83 ± 0.01^a	$1.18 \pm 2.45 \times 10^{-3}^b$	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 2. 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			

In the present study mean SOD values during summer was found 1.87 ± 0.10 in treatment group 1, $1.80 \pm 4.67E-03$ 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 $1.18 \pm 4.69E-03$ in treatment group 1, $1.18 \pm 4.00E-03$ in treatment group 2 and $1.19 \pm 3.95E-03$ in treatment group 3 with an aggregate of $1.18 \pm 2.45E-03^b$.

The present findings are in close proximity with the findings reported by Hasin (2015). She reported 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.

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 (Bernabucchi *et al.*, 2002). Superoxide dismutase, catalase and glutathione peroxidase are the major defense in the detoxification of superoxide anion and hydrogen peroxide (McCord *et al.*, 1969 and Chance *et al.*, 1979). Superoxide dismutase along with catalase and glutathione peroxidase scavenges both intracellular and extracellular

superoxide radicals and prevents lipid peroxidation (Agarwal and Prabhakaran, 2005). Superoxide dismutase that catalyzes dismutation of superoxide becomes important in the defense mechanisms against oxidative stress (Halliwell and Chirico, 1993). The SOD activity in summer was lowest in the melatonin supplemented group i.e., treatment 2. Al-Badwi *et al.*, (2013) suggested decrease SOD activity is due to consumption of SOD to overcome the oxidative stress. This might also be due to the antioxidant effects of melatonin that scavenges the free radicals generated during heat stress (Ahmed *et al.*, 2005). 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.

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