Thermal Stress Induced Catalase Activity Level in Selected Bivoltine Breeds of Mulberry Silkworm *Bombyx mori* L.

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Abstract

In order to understanding the importance and level of changes in catalase activity in the three body tissues of silkworm *Bombyx mori* L. under high temperature conditions, the larvae of five bivoltine breeds were exposed to 35±1°C and 40±1°C, during fifth instar. Based on the results in the present investigation, Catalase, significantly ($p<0.001$) revealed the highest level of activity in the fat body tissue, compared to midgut and haemolymph. As well as, compared to CSR_4, JROP, NB_4D_2 and KA breeds, CSR_2 relatively showed the greater level of catalase activity under the control (28±1°C) and imposed thermal stresses.

Keywords: Bivoltine breeds, *Bombyx mori* L., Catalase activity, High temperature

1. Introduction

Temperature plays a major role on the growth and productivity of silkworms, as the silkworm is poikilothermic (Benchamin and Jolly, 1986). There is ample literature showing that good quality cocoons are produced within a temperature range of 22 - 27°C and levels above these makes the cocoon quality worse (Krisnaswami et al., 1973). The effect of higher temperatures on silkworm larvae was reported earlier by Takeuchi et al. (1964) and Ohi and Yamashita (1977). India being a tropical country, temperature shoots up in the day time. In summer it goes up to 35 to 40°C or even more. These fluctuations in temperature have an adverse effect on the survival and pupation of silkworm, especially the bivoltine breeds, incurring heavy loss to the industry. Many of the silkworm characters are not only controlled by genes but also influenced by environmental factors such as temperature (Watanabe, 1918, 1919, 1924, 1928 & Kogure, 1933).

Earlier reports suggested that environmental stresses diminish *in vivo* antioxidant status and cause oxidative stress in living organism (Klasing, 1998 & Sahin et al., 2001). Oxidative stress is the result of an imbalance between pro-oxidant species and the levels of the defences resulting from the generation of reactive oxygen species (ROS) (Santoro & Thiele, 1997). Living organisms need mechanisms regulating reactive oxygen species (ROS) such as hydrogen peroxide and superoxide anion. Catalase (CAT) (H_2O_2:H_2O_2 oxidoreductase, EC 1.11.1.6, CAT) is one of the antioxidant enzymes and catalyzes the degradation of H_2O_2 to water and oxygen (Switala & Loewen, 2002). In chickens, oxidative stress was observed on exposure to acute heat stress (Mujahid et al., 2005 & Lin et al., 2006). In fact, ROS is harmful to living organisms because ROS tends to give oxidative damages to proteins, nucleic acids, and lipids (Hermes-Lima & Zenteno-Savı´n, 2002). In this context, ROS has been recognized to be related to aging and life span (Sohal et al., 1990, Orr and Sohal, 1994 and Parkes et al., 1999). On the other hand, ROS plays a helpful role in the innate immunity system of an insect (Hao et al., 2003 & Kumar et al., 2003). In insects, CAT is recognized as the key enzyme to be solely responsible for the scavenger of ROS (Felton & Summers, 1995). However, there is little information on insect CAT especially on its biochemical properties.

High temperature affects nearly all biological processes including the rates of biochemical and physiological reactions (Hazel, 1995 & Willmer et al., 2004), and it eventually can affect on the quality or quantity of cocoon crops in the silkworm. Several reports (Ueda & Lizuka, 1962, Shirota, 1992 & Tazima & Ohuma, 1995) demonstrated that silkworms were more sensitive to high temperature during 4^{th} and 5^{th} stages which are

recommended for the recognition and selection of thermotolerant silkworm breeds, under high temperature conditions.

In the present investigation, the main objective was to compare the level of changes in the catalyse activity in the three body tissues (fat body, midgut and haemolymph) of five popular bivoltine breeds under the control and imposed thermal stress conditions during the 5th instar larvae of the silkworm.

2. Research material and methods

Five bivoltine silkworm genotypes with higher cocoon productivity traits (Datta et al., $2000_{a,b}$) and known for higher temperature and disease tolerance (Mano, 1994) namely JROP, KA, NB_4D_2, CSR_2 and CSR_4, which were drawn from the germplasm bank of the Department of Sericulture Science, University of Mysore, Mysore, India during 2009. The rearing was conducted during monsoon following the method suggested by Krishnaswami (1978). The larvae were fed with an M_5 variety of mulberry (*Morus alba*) leaves. The breeds were considered with three replications at each temperature and each replicate included 250 larvae. They were divided within experimental trays at a rearing house based on a random completely design.

2.1 Temperature treatments

The control treatment was the ambient temperature (28±1°C) and based on summer temperature in tropical countries, which can rise to about 35-40°C in the day time two imposed temperatures of 35±1°C and 40±1°C were treated using BOD (Biological Oxygen Demand) incubator. The larvae were incubated twice a day for two hours, alternately with intervals of four hours. The exposing duration to thermal stress was started from the first to fifth days of 5th instar. Appropriate plastic boxes (25 × 18 × 7 cm in size) with a net lid were made and used for transferring larvae from the rearing house to BOD. After the heat treatment the tested larvae were transferred to ordinary rearing conditions at 28±1°C. All experimental silkworms in both control and treated batches were not fed during incubation and both were fed fresh mulberry leaves twice a day, 15 minutes after the stopping point of BOD. The humidity in BOD was adjusted equal to the rearing house humidity (75±2 %), using wet pads.

2.2 Tissue preparations

The tissue preparations were made on the 5th day of the 5th instar in both control and high temperature treated batches. To prepare the tissues for catalase activity estimation, haemolymph samples were collected from 6-7 larvae by random selection and by amputating one of the thoracic legs of the larvae in the prechilled centrifuge tube. The larvae were dissected in 0.9% saline at pH 6.5 on a chilled dissection tray, fat body and midgut, cleanly collected and stored at -20ºC. From each larva, 0.5ml haemolymph was extracted and to avoid the activity of prophenol oxidase followed by melanization of haemolymph, 1mg phenylthiourea was added to the haemolymph samples immediately after extraction. Then they were centrifuged for 10 min in 4000 rpm at -4ºC. The supernatant was transferred to new prechilled tubes and kept in -20ºC until the beginning of the chemical experiments.

2.3 Measurements of catalase activity

0.1 ml stored haemolymph; 100 mg fat body and 100 mg midgut were homogenized in 10ml of distilled water at -4ºC as the test solution. Total CAT activity was spectrophotometrically measured by the method of Aebi (1984). The decrease in absorbance at 240 nm was monitored at 30°C. To examine the distribution of the activity, the test solution was prepared by the following method: frozen tissues were homogenized in 70mM potassium phosphate buffer (pH 6.5) containing 0.1% Triton X-100, and insoluble substances were centrifuged out. Tissue protein concentrations were measured according to Lowry's method (1951). Each measurement was considered with 6 replicates.

2.4 Statistical analysis

Data were analysed using one-way ANOVA of SAS 9, when significant differences were found, means were separated. Comparisons of the treatment means were performed with Duncan's test (Duncan, 1955).

3. Results

There was a great difference ($P<0.001$) in amount of CAT activity in the three different tissues and was observed relatively high activity in the fat body, midgut compared to haemolymph, respectively (Table1).

The catalase activity levels in the three different tissues showed significant difference in selected silkworm breeds under the thermal stress conditions (Table 2-4) which highest level and changes of CAT activity among all tissues were observed in fat body (Table 1&2). The catalase activity levels in the fat body of all breeds under the 35±1°C displayed a little increase compare to the control, but it was statistically significant only in NB_4D_2

breed (Table 2), furthermore, acute stress causes significant decrease in the fat body catalase activity in all breeds at 40±1°C compare to the control and 35±1°C.

Imposed high temperature did not show significant influence on midgut catalase activity in all breeds except JROP and NB_4D_2 (Table 3) which both revealed a significant decrease under 40±1°C.

The amount of changes in haemolymph catalase activity of JROP, KA and NB_4D_2 breeds was significant under high temperatures except in CSR_2 and CSR_4 breeds (Table 4), as well as, JROP, KA and NB_4D_2 breeds revealed a significant decrease under 40±1°C temperature.

In general, among all breeds, CSR_2 expressed the highest level of CAT activity in the three tissues, under the different temperature, especially at the 40±1°C thermal stress (Fig. 1-3).

4. Discussion

CAT in insects has been considered to be solely responsible for the scavengery of H_2O_2, because insects are deficient in a selenium-dependent glutathione peroxidase that is another scavenger present in other organisms (Ahmad & Pardini, 1990 & Sohal et al., 1990). There are however, few reports describing biochemical properties of insect CAT. In the present investigation, the activity of CAT was widely distributed in various tissues of *B. mori* larvae and it is found a great difference ($P<0.001$) in amount of CAT activity in three different tissues, as well as observed relatively high activity in the fat body, midgut and haemolymph, respectively (Table 1). This could happen due to more metabolism level in the fat body's tissue. It was reported that there was a notable difference in enzyme activities of CAT among tissues in the mouse and that the highest activity was detected in liver (Remmen et al., 1998). Similarly, cytochemical studies on CAT from the Brazilian skipper (*Calpodes ethlius*) revealed that CAT activity was present in fat body (Locke & McMahon, 1971). These results were interesting, because the fat body plays a physiological role similar to that of the liver in mammals (Keeley, 1985). Many authors described the physiological responses BmCAT (*Bombyx mori* catalase) also plays a significant role most probably in curing the oxidative stress by facilitating the degradation of H_2O_2. In this context, Yamamoto et al. (2005) exhibited that irradiation of gamma rays to *B. Mori* larvae causes a notable increase in CAT activity in the midgut and fat body. In the present study, all breeds revealed a significant decrease in the fat body CAT activity at 40±1°C (Table 2) and probably acute stress at 40±1°C can be considered as the main reason for this result. A similar result was obtained in midgut CAT activity in JROP and NB_4D_2 breeds but the CAT activity in remaining three breeds increased at 40±1°C same the results obtained in control condition (Table 3). Further, a same performance took place in the haemolymph CAT activity at the three breeds of JROP, NB_4D_2 and KA out of five breeds (Table 4). Similarly, Nagesh and Devaraj (2008) reported that catalase drastically reduced in temperature stress in French bean (*Phaseolus vulgaris*). Catalase activity is necessary for heat-shock recovery in *Aspergillus nidulans* germlings (Maria et al., 1999) and high temperature increase results in oxidative stress in goldfish *Carassius auratus* L. and activity of catalase decreased significantly (Lushchak Volodymyr & Bagnyukova Tetyana, 2006).

The haemolymph is cellular and componential liquid media mainly responsible for the interagral function of body to observe and maintain the physiological and metabolic status of the organism. Midgut tissue plays a key role in the processes of digestion, absorption and assimilation of digested nutritive component of mulberry leaf.

The growth and differentiation are closely related to protein synthesis during the 5^{th} instar which it is mainly depended to food intake and food intake reaches its peak in the 5^{th} instar in according with body growth and silk gland development and metabolism in the silkworm (Jolly, et al., 1974). Further, the fat body tissue is the seat of metabolism, active in the synthesis, storage and release of biomolecules to provide clues in relation to energy production, transformation and utilization at cellular and subcellular level during intermediary metabolism in silkworm body. As well, acute thermal stress at 40±1°C, negatively affected rate of food intake, protein synthesis and metabolism during the 5^{th} instar and due to these changes, probably, an intensive decrease was observed in the fat body catalase activity.

5. Conclusions

In general and with regard to CAT activities in different tissues which were obtained in the present investigation (Table 1-4) the highest activity of catalase was in fat body tissue. Furthermore, compared to CSR_4, JROP, NB_4D_2 and KA bivoltine breeds of mulberry silkworm, CSR_2 breed relatively showed the most level of catalase activity in the different tissues and under the different temperature regimes (Fig. 1-3).

However, to understand antioxidant mechanism in insects, more information on biochemical and physiological properties of insect CAT is required.

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References

Aebi, H. (1984). Catalase *in vitro*. *Method Enzymol*., 105, 121-126.

Ahmad, S., & Pardini, R. S. (1990). Mechanisms for regulating oxygen toxicity in phytophagous insects. *Free Radic. Biol. Med*., 8, 401-413.

Benchamin, K. V., & Jolly, M. S. (1986). Principles of silkworm rearing. *Proceeding of Seminar on Problems and Procpects of Sericulture*, S. Mahalingam (ED) (pp. 63-108) Vellore, India.

Datta, R. K., Basavaraja, H. K., Mal Reddy, N., Nirmal Kumar, S., Ahsan, M. M., Suresh Kumar, N., & Ramesh Babu, M. (2000_a). Evolution of new productive bivoltine hybrids $CSR_2 \times CSR_4$ and $CSR_2 \times CSR_5$. *Sericologia*, 40(1), 151-167.

Datta, R. K., Basavaraja, H. K., Mal Reddy, N., Nirmal Kumar, S., Ahsan, M. M., Suresh Kumar, N., & Ramesh Babu, M. (2000_b). Evolution of new productive bivoltine hybrid $CSR_3 \times CSR_6$. *Sericologia*, 40(3), 407-416.

Duncan, D. B. (1955). Multiple range and multiple F- tests. *Biometrics*, 11, 1-42.

Felton, G. W., & Summers, C. B. (1995). Antioxidant system in insects. *Arch. Insect. Biochem. Physiol*., 29, 187-197.

Hao, Z., Kasumba, I., & Aksoy, S. (2003). Proventriculus (cardia) plays a crucial role in immunity in tsetse fly (Diptera: Glossinidiae). *Insect Biochem. Molec. Biol*., 33, 1155-1164.

Hazel, J. R. (1995). Thermal adaptation in biological membranes: Is homeoviscous adaptation the explanation? Annu. *Rev. Physiol*., 57, 19-42.

Hermes-Lima, M., & Zenteno-Savı´n, T. (2002). Animal response to drastic changes in oxygen availability and physiological oxidative stress. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol*., 133, 537-556.

Jolly, M. S., Sen, S. K., & Ahsan, M. M. (1974). Tasar Culture (1st Ed*.). Central Silk Board, Bombay* (pp. 1-266), India.

Keeley, L. L. (1985). Physiology and biochemistry of the fat body. In: Kerkut, G. A., Gilbert, L. I. (Eds.). *Comprehensive Insect Physiology Biochemistry and Pharmacology Pergamon Press*, Oxford, 3, 211-248.

Klasing, K. C. (1998). *Comparative Avian Nutrition*. University Press, Cambridge.

Kogure, M. (1933). The influence of light and temperature on certain characters of the silkworm, *Bombyx mori*. *J. Dept. Agr. Kyushu. Imp. Univ*., 4, 1-93.

Krishnaswami, S., Narasimhanna, M. N., Suryanarayana, S. K., & Kumararaj, S. (1973). *Silkworm rearing* (pp. 53-90) Rome: Bulletin 15/2, FAO Agriculture Services, United Nations Organizations.

Krishnaswami, S. (1978). New technology of silkworm rearing. *Central Sericultural Research and Training Institute* (pp. 23), Mysore, India: Bulletin No. 2.

Kumar, S., Christophides, G. K., Cantera, R., Charles, B., Han, Y. S., Meister, S., Dimopoulos, G., Kafatos, F. C., & Barillas-Mury, C. (2003). The role of reactive oxygen species on Plasmodium melanotic encapsulation in Anopheles gambiae*. Proc. Natl. Acad. Sci*. USA, 100, 14139-14144.

Lin, H., Decuypere, E., & Buyse, J. (2006). Acute heat stress induces oxidative stress in broiler chickens. *Comp. Biochem. Physiol. A*, 144, 11-17.

Locke, M., & Mc Mahon, J. T. (1971). The origin and fate of microbodies in the fat body of an insect. *J. Cell Biol*., 48, 61-78.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). *J. Biol. Chem*., 193, 265 (The original method).

Lushchak Volodymyr, I., & Bagnyukova Tetyana, V. (2006). Temperature increase results in oxidative stress in goldfish tissues. 2. Antioxidant and associated enzymes. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 143, 36-41.

Maria, A. N-J., Ricardo, M. C., Maria, H. S. G., Jesus, A., Suresh, I., Allan, C., Hector, F. T., & Gustavo, H. G. (1999). Catalase activity is necessary for heat-shock recovery in Aspergillus nidulans germlings. *Microbiology*, 145, 3229-3234.

Mano, Y. (1994). A comprehensive report on silkworm breeding (pp. 101). Bangalore, India: submitted to CSB.

Mujahid, A., Yoshiki, Y., Akiba, Y., & Toyomizu, M. (2005_b). Superoxide radical production in chicken skeletal muscle induced by acute heat stress. *Poult. Sci*., 84, 307-314.

Nagesh Babu, R., & Devaraj, V. R. (2008). High temperature and salt stress response in French bean (*Phaseolus vulgaris*). *Australian Journal of Crop Science*, 2(2), 40-48.

Ohi, H., & Yamashita, A. (1977). On the breeding of the silkworm races J137 and C137. *Bull. Seric. Exp. Stn.*, 27, 97-139.

Orr, W. C., & Sohal, R. S. (1994). Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. *Science*, 263, 1128-1130.

Parkes, T. L., Hilliker, A. J., & Phillips, J. P. (1999). Motorneurons, reactive oxygen, and life span in Drosophila. *Neurobiol Aging.*, 20, 531-535.

Remmen, H. V., Williams, M. D., Yang, H., Walter, C. A., & Richardson, A. (1998). Analysis of the transcriptional activity of the 50-flanking region of the rat catalase gene in transiently transfected cells and in transgenic mice. *J. Cell Physiol*., 174, 18-26.

Sahin, K., Sahin, N., Onderci, M., Yarahoglu, S., & Kucuk, O. (2001). Protective role of supplemental Vitamin E on lipid peroxidation, Vitamins E, A and some mineral concentrations of broilers reared under heat stress. *Vet. Med. Czech*., 46,140-4.

Santoro, N., & Thiele, D. J. (1997). Oxidative stress responses in the yeast. *Yeast Stress Responses* (pp. 171-211). Springer.

Shirota, T. (1992). Selection of healthy silkworm strain through high temperature rearing of fifth instar larvae. *Reports of the Silk Science Research Institute*, 40, 33-40.

Sohal, R. S., Arnold, L., & Orr, W. C. (1990). Effect of age on superoxide dismutase, catalase, glutathione reductase, inorganic peroxidases, TBA-reactive material, GSH/GSSG, NADPH/NADP+ and NADH/NAD+ in Drosophila melanogaster. *Mech. Ageing. Dev*., 56, 223-235.

Switala, J., & Loewen, P. C. (2002). Diversity of properties among catalases. *Arch. Biochem. Biophys*., 401, 145-154.

Takeuchi, Y., Kosaka, T., & Ueda, S. (1964). The effects of rearing temperature upon the amount of food ingested and digested. *Tech. Bull. Seric. Exp. Stn*., 84, 1-12.

Tazima, Y., & Ohnuma, A. (1995). Preliminary experiments on the breeding procedure for synthesizing a high temperature resistant commercial strain of the silkworm, *Bombyx mori* L. *Silk Sci. Res. Inst. Jpn*., 43, 1-16.

Ueda, S., & Lizuka, H. (1962). Studies on the effects of rearing temperature affecting the health of silkworm larvae upon the quality of cocoons - I. Effect of temperature in each instar. *Acta Sericologia Jpn*., 41(4), 6-21.

Watanabe, K. (1918). Studies on the voltinism in the silkworm. I. Inheritance of bi and tetravoltine characters. *Sanshi Shikenjo Hokoku*, 3, 397- 437.

Watanabe, K. (1919). Studies on the voltinism in the silkworm Bombyx mori L. I. Inheritance of univoltine vs. multivoltine. *Bull. Seric. Expt. Stn. Jpn*., 4, 87-106.

Watanabe, K. (1924). Studies on the voltinism in the silkworm. *Sanshi Shikenjo Hokoku*, 6, 411-435.

Watanabe, K. (1928). Further Studies on the voltinism in the silkworm. *Bombyx mori* L. *Bull. Seric. Expt. Stn. Jpn*., 7, 285-303.

Willmer, C. W., Stone, G., & Johnston, I. (2004). Environmental physiology of animals. *Blackwell Science*, Oxford.

Yamamoto, K., Zhang, P., Banno, Y., Fugii, H., Miake, F., Kashige N., & Aso, Y. (2005). Superoxide dismutase from the silkworm, *Bombyx mori*: sequence, distribution and overexpression. *Biosci. Biotechnol. Biochem*., 69 (3), 507-514.

Table 1. Changes in the level of catalase activity in the three different tissues under the different temperature regimes

Tissue	Temperature		
	Control (28±1°C)	35±1°C	40±1°C
Fat body	6.34 [a]	6.96 [a]	5.22 [a]
Midgut	5.29 [b]	5.49 [b]	4.78 [b]
Haemolymph	3.63 [c]	3.69 [c]	3.00 [c]

Means having the different superscript letters differ significantly (P<0.001).

Table 2. Mean squares and changes in the level of CAT activities in fat body under the different temperature regimes (Units/mg protein) (Each value is the mean ± SD of 6 separate replications)

Temperature	CSR_4	CSR_2	JROP	KA	NB_4D_2
28±1ºC (Control)	6.125 ± 0.166 [a]	6.846 ± 0.157 [ab]	6.478 ± 0.276 [ab]	6.408 ± 0.198 [a]	5.822 ± 0.143 [b]
35±1ºC	6.694 ± 0.320 [a]	7.444 ± 0.232 [a]	6.787 ± 0.497 [a]	6.995 ± 0.076 [a]	6.861 ± 0.308 [a]
40±1ºC	4.719 ± 0.179 [b]	6.047 ± 0.339 [b]	5.243 ± 0.126 [b]	5.364 ± 0.190 [b]	4.745 ± 0.167 [c]
Significance level	**	*	*	**	**

Means having the same subscript letters do not differ significantly

* and ** = Significant at 0.005 and 0.001 level of probability, respectively

Table 3. Mean squares and changes in the level of CAT activities in midgut under the different temperature regimes (Units/mg protein) (Each value is the mean ± SD of 6 separate replications)

Temperature	CSR_4	CSR_2	JROP	KA	NB_4D_2
28±1ºC (Control)	5.100 ± 0.121	5.656 ± 0.213	5.400 ± 0.244 [ab]	5.212 ± 0.397	5.100 ± 0.120 [ab]
35±1ºC	5.056 ± 0.010	5.640 ± 0.322	5.746 ± 0.091 [a]	5.437 ± 0.245	5.556 ± 0.234 [a]
40±1ºC	4.662 ± 0.106	5.280 ± 0.247	4.665 ± 0.323 [b]	4.776 ± 0.176	4.538 ± 0.273 [b]
Significance level	ns	ns	*	ns	*

Means having the same subscript letters do not differ significantly

* = Significant at 0.005 level of probability

ns = Not statistically significant

Table 4. Mean squares and changes in the level of CAT activities in haemolymph under the different temperature regimes (Units/mg protein) (Each value is the mean ± SD of 6 separate replications)

Temperature	CSR_4	CSR_2	JROP	KA	NB_4D_2
28±1ºC (Control)	3.517 ± 0.250	3.941 ± 0.060	3.494 ± 0.176 ^{ab}	3.831 ± 0.156 ^{a}	3.388 ± 200 ^{ab}
35±1ºC	3.240 ± 0.272	4.016 ± 0.60	3.776 ± 0.141 ^{a}	3.778 ± 0.283 ^{a}	3.647 ± 0.090 ^{a}
40±1ºC	2.611 ± 0.205	3.792 ± 0.157	3.072 ± 0.094 ^{b}	2.672 ± 0.325 ^{b}	2.849 ± 0.122 ^{b}
Significance level	ns	ns	*	*	*

Means having the same subscript letters do not differ significantly

* = Significant at 0.005 level of probability, respectively

ns = Not statistically significant

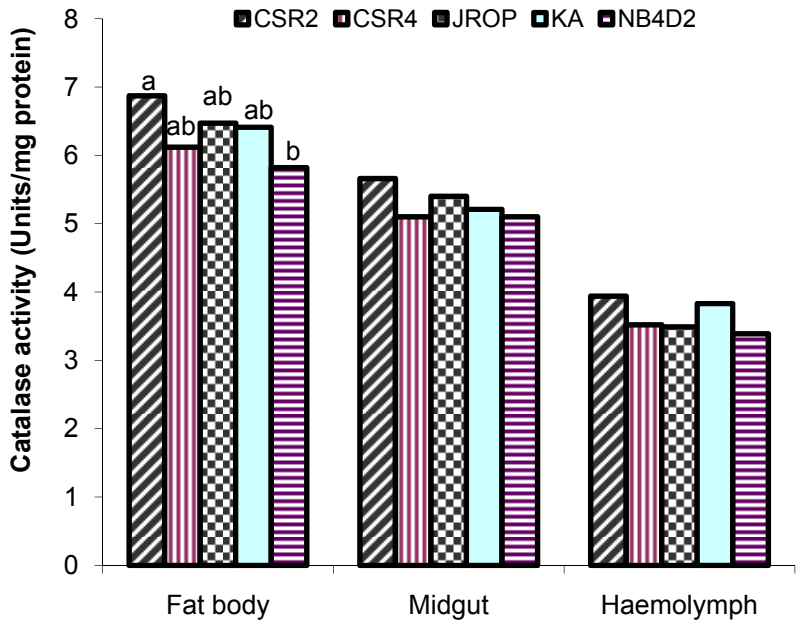

Figure 1. Catalase activity of different breeds in the three tissues under 28±1ºC (Control)
(Columns having the different letters differ significantly ($p<0.05$), for each tissue separately)

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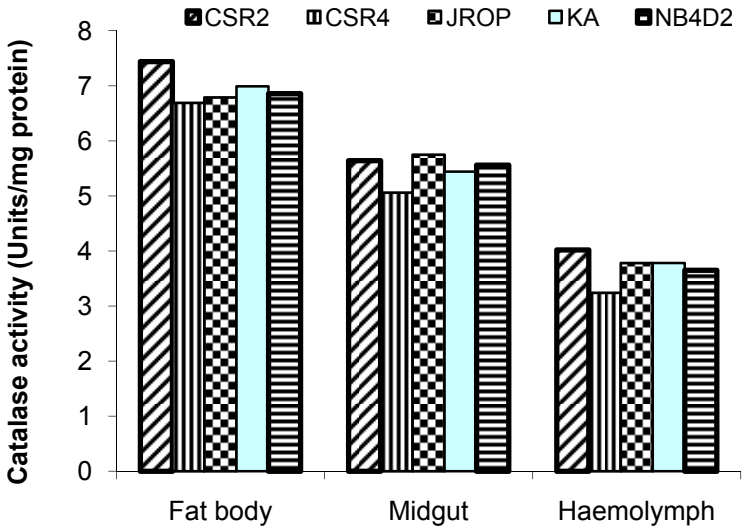

Figure 2. Catalase activity of different breeds in the three tissues under 35±1ºC (Control)

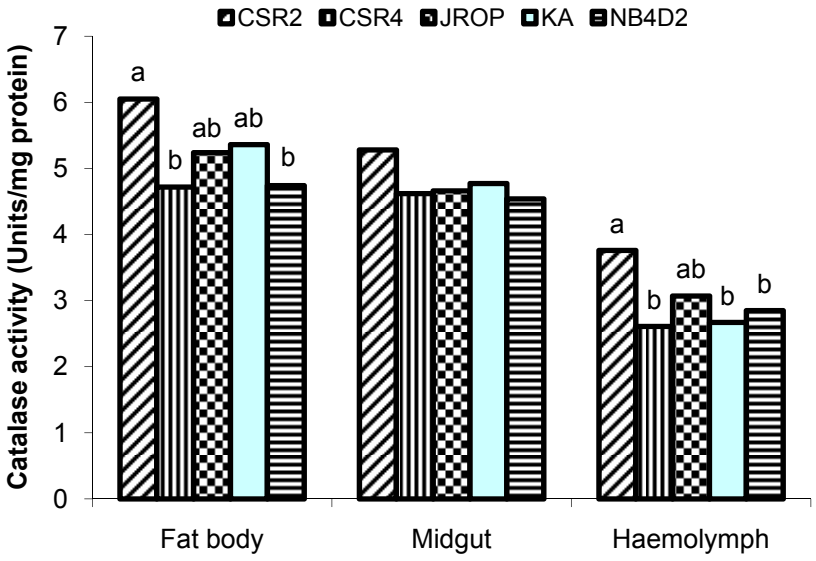

Figure 3. Catalase activity of different breeds in the three tissues under 40±1ºC (Control)
(Columns having the different letters differ significantly ($p<0.05$), for each tissue separately)