10-2015

SHORT COMMUNICATION: EFFECT OF SACCHAROMYCES CEREVISIAE SUPPLEMENTATION ON SOME BIOMARKERS OF OXIDATIVE STRESS IN WEANED RABBITS DURING THE HOT-DRY SEASON

B.M. Shehu

B.A. Ayanwale

J.O. Ayo

C. Uchendu

World Rabbit Science

World Rabbit Sci. 2016, 24: 67-70
doi:10.4995/wrs.2016.1656

SHORT COMMUNICATION: EFFECT OF *SACCHAROMYCES CEREVISIAE* SUPPLEMENTATION ON SOME BIOMARKERS OF OXIDATIVE STRESS IN WEANED RABBITS DURING THE HOT-DRY SEASON

SHEHU B.M.*†, AYANWALE B.A.‡, AYO J.O.#, UCHENDU C.#

*Department of Agricultural Leadership, Education and Communication, University of Nebraska-Lincoln, United States of America.
†National Agricultural Extension and Research Liaison Services, Ahmadu Bello University, Zaria, Nigeria.
‡Department of Animal Production, Federal University of Technology, Minna, Nigeria.
#Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, Nigeria.

Abstract: A feeding trial was conducted to evaluate the effect of *Saccharomyces cerevisiae* (SC) supplementation on some biomarkers of oxidative stress in rabbits during the hot-dry season (temperature-humidity index: 33.2±0.9ºC). Sixty healthy weaned crossbred rabbits, aged between 5-6 wk with live weight of 612.7±60.8 g (mean±standard deviation) were used. The rabbits were divided into 5 treatment groups; SC0 receiving a control diet without supplementation of SC, and SC2, SC4, SC6 and SC8 receiving the control diet supplemented with SC at the rate of 2, 4, 6 and 8×10^9 colony forming units/kg, respectively. The activity of total superoxide dismutase and malonaldehyde in serum were not significantly affected, but serum catalase concentration rose ($P<0.05$) as the SC inclusion level increased. Although further studies are required, baker's yeast containing SC could help ameliorate the adverse effects of heat stress in rabbits.

Key Words: *Saccharomyces cerevisiae*, oxidative stress, rabbits, hot-dry season.

INTRODUCTION

Rabbit production provides the impoverished urban population and rural dwellers with opportunities to earn additional income on a sustainable basis. Thermal environmental conditions of high ambient temperature (AT) and high relative humidity (RH), characteristic of the hot-dry season in Nigeria, cause heat stress, which exerts adverse effects on livestock production (Dzenda *et al.*, 2011), and has been described as thermally stressful to livestock (Ayo *et al.*, 1996, 1998; Dzenda *et al.*, 2011). The thermo-neutral zone (TNZ) of rabbits in the tropics is 18-21°C (Marai and Habeeb, 1994; Habeeb *et al.*, 1998). The influence of high AT on the body temperature of rabbits is a limiting factor for their performance in warm countries (Marai *et al.*, 2002; Cervera and Fernández-Carmona, 2010).

Oxidative processes in animals, including those induced by thermal stress, result in the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Vandana *et al.*, 2006), which may result in cellular damage and decreased activities of antioxidant enzymes and concentration of non-enzymatic antioxidant molecules, which may lead to the development of diseases of economic importance (Vandana *et al.*, 2006; Orrenius *et al.*, 2007; Niki, 2009). Alleviating oxidative stress in rabbits includes the administration of antioxidants to supplement the endogenous antioxidants (Zeweil *et al.*,2013; Simitzis *et al.*, 2014; Trebušak *et al.*, 2014), especially in thermal stress situations (El-Hanoun *et al.*, 2014). Yeasts and their extracts are sources of natural antioxidants (Nishino and Ishikawa, 1998; Gazi *et al.*, 2001). Therefore, the present study was undertaken to investigate the effect of varying

Correspondence: Shehu BM, bshehu@huskers.unl.edu. Received *August 2013* - Accepted *October 2015.*
http://dx.doi.org/10.4995/wrs.2016.1656

levels of baker's yeast, *Saccharomyces cerevisiae* (SC) supplementation on some serum biomarkers of oxidative stress in weaner rabbits during the hot-dry season at the Northern Guinea Savannah zone of Nigeria.

MATERIAL AND METHODS

Study area and thermal environmental conditions

The study was conducted at the rabbitry unit of the Skill Acquisition and Development Centre of the National Agricultural Extension and Research Liaison Services (NAERLS), Ahmadu Bello University, Zaria (11°12' N, 07°33' E), located in the Northern Guinea Savannah zone of Nigeria. The experiment was conducted during the hot dry season (March to April, 2011), frequently described as thermally stressful to livestock (Ayo *et al.*, 1996, 1998; Sinkalu *et al.*, 2009). Wet- and dry-bulb temperatures of the rabbitry were recorded once a week at 6:00, 13:00 and 18:00 h during the experimental period, using wet- and dry-bulb thermometers. The RH was calculated from the values of wet- and dry-bulb temperature according to the manufacturer's manual attached. Temperature-humidity index (THI) was calculated using the method of Marai *et al.* (2002) for rabbits:

$$THI: db–[(0.31–0.31(RH)\ (db–14.4)]$$

where db=dry-bulb temperature (in degrees Celsius) and RH=relative humidity percentage/100. The values obtained for THI were then classified as follows (Marai *et al.*, 2002): <27.8=absence of heat stress, 27.8-28.9=moderate heat stress, 28.9-30.0 severe heat stress and 30.0 and more=very severe heat stress.

Experimental animals, diets and procedures

Sixty healthy weaned crossbred rabbits of both sexes, aged between 5-6 wk with live weight of 612.7±60.8 g (mean±standard deviation) were procured from National Animal Production Research Institute (NAPRI), Shika-Zaria, Nigeria. The rabbits were divided by simple randomisation into 5 groups of 12 animals per treatment, after balancing for live weight: SC0 receiving a control diet without supplementation of SC, and SC2, SC4, SC6 and SC8 receiving the control diet supplemented with SC at the rate of 20, 40, 60 and 80 g per kg for 8 wk (corresponding to 0, 2, 4, 6 and 8×10^9 colony-forming unit/kg, respectively). A commercial baker's yeast, Vahine® (Avignon, Monteux, France), containing SC was used for the dietary supplementation. Proximate analysis of the basal mixture (control diet; SC0) which contained maize, soybean, maize offal, brewer's dried grain, groundnut cake, blood meal, rice offal and bone meal as main ingredients, showed that it contained 16.0% crude protein, 14.1% crude fibre, 3.9% ether extract, and 10.2% ash/kg feed. The rabbits were housed in a well-ventilated rabbitry in 3 tier-wire cages. Each cage measured 70×60×50 cm in length, width and height, respectively. The experiment lasted 56 d.

Blood sample collection and serum evaluation for biomarkers of oxidative stress

At the end of the feeding period, 6 rabbits from each treatment (2 from each replicate) were starved overnight of feed for 12 h before blood samples were collected. Blood sample (3 mL) was collected aseptically from each rabbit from the marginal vein of the ear using a sterilised disposable syringe and needle between 06:30 and 07:30 a.m. The blood sample was transferred into a centrifuge tube, allowed to clot and then incubated for 30 min, thereafter centrifuged at 2000 *g* for 10 min in a microcentrifuge to obtain serum. The serum samples obtained were used to determine the activities of superoxide dismutase (SOD) and catalase (CAT), and malonaldehyde (MDA) concentration. Total SOD activity was measured using the method described by Misra and Fridovich (1972), while that of CAT was determined according to the method adapted from Beers and Sizer (1952). Serum MDA concentration was determined by the thiobarbituric acid (TBA) assay: Briefly, a sample of 0.50 mL of serum was added to 3 mL of 1% phosphoric acid, 1 mL of 0.060% TBA, and 0.15 mL of 0.20% butylated hydroxytoluene in 95% methanol. The samples were heated in a boiling water bath for 45 min, cooled and 4 mL of 1-butanol was added. The butanol phase was separated by centrifugation at 3000 *g* for 10 min and the absorbance measured using a UV spectrophotometer (Jenway, 6405 model, Japan) at 535 nm. The concentration of MDA was calculated and expressed as mmol/L according to the procedure of Dandekar *et al.* (2002).

Statistical analysis

The data obtained were subjected to one-way ANOVA test in a completely randomised design using SAS 9.1 software package (SAS Institute, 2004), with the type of diet SC level serving as the main source of variation. The means were compared using Duncan's New Multiple Range Test (Duncan, 1955). Tests for polynomial orthogonal contrasts (linear, quadratic, cubic and quartic) were applied for the different supplemental levels of *Saccharomyces cerevisiae*. Statistical significance was set at $P<0.05$.

RESULTS

The period was characterised by high AT and RH values: on av. 34.4±1.1°C (range: 30-38.5°C) and 81.2±2.6% (range: 69.5-93%), respectively. The average THI obtained during the period was 33.2±0.9, indicating exposure of the animals to very severe heat stress. Serum SOD activity was not significantly affected by the level of supplemented SC (Table 1). CAT activity rose significantly ($P<0.05$) across the treatment groups as the SC inclusion level increased. There was no significant change ($P>0.05$) in MDA concentrations recorded across the treatment groups. Polynomial orthogonal contrasts showed a significant linear trend ($P<0.01$) only for CAT activity.

DISCUSSION

Although the proximate mechanism of action of SC as an antioxidant was not elucidated in the present study, yeasts and their extracts are sources of natural antioxidant compounds (Nishino and Ishikawa, 1998; Gazi *et al.*, 2001). In the present study, there was no significant increase in SOD activity ($P>0.05$) obtained due to yeast supplementation (although values registered increased with the SC supplementation). CAT is a key component of the antioxidant defence system. It plays an important role in the elimination of H_2O_2, furthering its catalysis into H_2O and O_2^-. Inhibition of the protective mechanism results in enhanced sensitivity to free radical-induced cellular damage in the body. Therefore, the reduction in the activity of this enzyme may result in a number of deleterious effects due to accumulation of O_2^- radicals and H_2O_2 (Vandana *et al.*, 2006). Supplementing rabbit diet with yeast culture increased ($P<0.05$) the activities of CAT, thereby preventing the accumulation of excessive ROS. Gutowicz *et al.* (2008) and Lecewicz *et al.* (2008) in birds reported that an increase in the activity of CAT in the blood is caused by environmental burdens to which they are exposed to during their growth. In addition, growth processes in early life are characterised by the generation of ROS through cellular division and apoptosis. This is because ROS are considered the major mediators of oxygen cytotoxicity and are important messengers stimulating cell division and manifesting cellular signalling effects (Buetler *et al.*, 2004). The findings of the present study indicate that yeast supplementation decreased oxidative stress and, consequently, ROS-mediated tissue damage by increasing the activity of CAT to cope with the increased ROS generated growth and other processes. There was a non-significant ($P>0.05$) reduction in serum MDA concentration in the rabbits fed SC-supplemented diet. The reduction could suggest a decrease in lipid peroxidation and the ability of yeast as an antioxidant in the defence mechanism to prevent the formation of excessive free radicals in the body.

Table 1: Serum antioxidant enzyme activities and malonaldehyde concentration of weaned rabbits fed varying levels of *Saccharomyces cerevisiae* (SC) supplemented diets (n = 30).

	Dietary treatments[1]					
	SC0	SC2	SC4	SC6	SC8	*P*-value
SOD (U/mL)	1.58±0.12	1.62±0.14	1.65±0.07	1.70±0.10	1.75±0.08	NS
CAT (U/mL)	40.33±1.20[a]	45.83±1.14[b]	46.17±0.95[b]	46.50±2.54[b]	48.50±0.72[b]	$P<0.05$
MDA (mmol/L)	2.42±0.20	2.38±0.28	2.32±0.07	2.27±0.15	2.27±0.09	NS

[1]SC0, SC2, SC4, SC6 and SC8 contained 0, 2, 4, 6 and $8 \cdot 10^9$ colony-forming units of SC per kg, respectively.

[ab]Means in the same row with different superscript letters are significantly ($P<0.05$) different.

SOD: superoxide dismutase; CAT: Catalase; MDA: Malonaldehyde; NS: Not significant.

CONCLUSIONS

The present study has shown that supplementation of weaned rabbit diets with baker's yeast containing SC could help ameliorate the adverse oxidative effects associated with heat stress conditions, as shown by the levels of the oxidative stress biomarkers studied. Further studies on other biomarkers are nevertheless required.

Acknowledgements: The authors gratefully acknowledge the Tertiary Education Fund of Nigeria (TETFund) for partially financing this study. Thanks also to the staff of rabbitry unit of the Skill Acquisition and Development Centre of the National Agricultural Extension and Research Liaison Services (NAERLS), Ahmadu Bello University, Zaria.

REFERENCES

Ayo J.O. Oladele S.B, Fayomi A. 1996. Effects of heat stress on livestock production: A review. *Nig. Vet. J., 1(Spec. Ed.): 49-57.*

Ayo J.O., Oladele S.B., Ngam S., Afolayan S.B., Fayomi A. 1998. Diurnal variation of rectal temperature of Red Sokoto goats during the harmatttam season. *Res. Vet. Sci., 66: 7-9. doi:10.1053/rvsc.1998.0231*

Beers R.F, Sizer I.W. 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by Catalase. *J. Biol. Chem., 195: 130-140.*

Buetler T.M., Krauskopf A., Ruegg U.T. 2004. Role of superoxide as a signalling molecule. *News Physiol. Sci., 19: 120-123. doi:10.1152/nips.01514.2003*

Cervera C., Fernández-Carmona J. 2010. Nutrition and the climatic environment. *In: De Blas C, Wiseman J, editors. Nutrition of the Rabbit, Wallingford, UK: CABI Publishing, 267-284. doi:10.1079/9781845936693.0267*

Dandekar S.P., Nadcarni G.D., Kulkarni V.S., Punekar S. 2002. Lipid peroxidation and antioxidant enzymes in male infertility. *J. Postgrad. Med., 48: 186-189.*

Dzenda T., Ayo J.O., Lakpini C.A.M., Adelaiye A.B. 2011. Seasonal and sex variations in live weights of captive African Giant rats (*Cricetomys gambianus*, Waterhouse) in the Northern Guinea Savanah zone of Nigeria. *Int. J. Zool. Res., 7: 49-58.*

Duncan D.B. 1955. New multiple range and multiple F-test. *Biometrics, 11: 1-42. doi:10.2307/3001478*

El-Hanoun A., Elkomy A., Fares W., Shahien E. 2014. Impact of royal jelly to improve reproductive performance of male rabbits under hot summer conditions. *World Rabbit Sci., 22: 241-248. doi:10.4995/wrs.2014.1677*

Gazi M.R., Hoshikuma A., Kanda K., Murata A., Kato F. 2001. Detection of free radical scavenging activity in yeast culture. *Saga Daigaku Nogakubu Iho, 86: 67-74.*

Gutowicz M., Cholojczyk M., Pyrzanowska R.M., Cholojczyk M., Pyrzanowska J., Widy-Tyszkiewicz E., Baranczyk-Kuzma A. 2008. Effect of curcumin on antioxidant and detoxification mechanisms in the livers of aging rats. *Med. Weter, 64: 955-957 (in Polish).*

Habeeb A.A.M., El-Maghawry A.M., Marai I.F.M., Gad A.E. 1998. Physiological thermoregulation mechanism in rabbits drinking saline water under hot summer conditions. *In Proc.: 1st International Conference on Indigenous versus Acclimatized Rabbits, 7- 9 September 1998. El-Arish, North Sinai, Egypt, pp. 443-456.*

Lecewicz A., Jankowski J., Zdunczyk Z., Juskiewicz J. 2008. Selected factors stimulating the development of some gastrointestinal parts in turkeys. *Med. Weter, 64: 1184-1186.*

Marai I.F.M., Habeeb A.A.M. 1994. Thermoregulation in rabbits. *Options Mediterr., 8: 33-41.*

Marai I.F.M., Habeeb A.A.M., Gad A.E. 2002. Rabbits' productive, reproductive and physiological performance traits as affected by heat stress: a review. *Livest. Prod. Sci., 78: 71-90. doi:10.1016/S0301-6226(02)00091-X*

Misra H.P., Fridovich I. 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem., 247: 3170-3175.*

Niki E. 2009. Lipid peroxidation, physiological levels and dual biological effects. *Free Radic. Biol. Med., 47: 469-484. doi:10.1016/j.freeradbiomed.2009.05.032*

Nishino T., Ishikawa F. 1998. Pharmaceutical, cosmetic, and food antioxidants containing yeasts and a method for evaluation of antioxidant activity of microorganisms. *Japan Patent, 10287872 A2 19881027.*

Orrenius S., Gogvadze V., Zhivotovsky B. 2007. Mitochondrial oxidative stress: Implications for cell death. *Ann. Rev. Pharmacol. Toxicol., 47: 143-183. doi:10.1146/annurev.pharmtox.47.120505.105122*

SAS. 2004. SAS/STAT User's Guide. *SAS Inst. Inc., Cary NC, USA.*

Simitzis P., Babaliaris C., Charismiadou M., Papadomichelakis G., Goliomytis M., Symeon G., Deligeorgis S. 2014. Effect of hesperidin dietary supplementation on growth performance, carcass traits and meat quality of rabbits. *World Rabbit Sci., 22: 113-121. doi:10.4995/wrs.2014.1760*

Sinkalu V.O., Ayo J.O., Adelaiye A.B., Hambolu J.O. 2009. Influence of co-administration of vitamins A and E on diurnal variation in rectal temperature of Black Harco pullets during the hot - dry season. *Biol. Rhythm Res., 40: 257-264. doi:10.1080/09291010701736991*

Trebušak T., Levart A., Frankič T., Pirman T. 2014. Effect of dietary linseed oil and *Ganoderma lucidum* or olive leaves supplementation on fatty acid composition and oxidative status of rabbits. *World Rabbit Sci., 22: 71-81. doi:10.4995/wrs.2014.1183*

Vandana S., Ram S., Ilavazhagan M., Kumar G.D., Banerjee P.K., 2006. Comparative cytoprotective activity of vitamins C, E and beta-carotene against chromium-induced oxidative stress in murine macrophages. *Biomed. and Pharmacother., 60: 71-76. doi:10.1016/j.biopha.2005.04.005*

Zeweil H., Elnagar S., Zahran S., Ahmed M., El-Gindy Y. 2013. Pomegranate peel as a natural antioxidant boosts bucks' fertility under Egyptian summer conditions. *World Rabbit Sci., 21: 33-39. doi:10.4995/wrs.2013.1209*