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Effect of Dietary Supplementation with Rosemary Essential Oil and Selenium on Fertility Gene Expression in Broiler Breeder Roosters

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Abstract

Advancing age in broiler roosters reduces reproductive potential. Natural antioxidants, such as rosemary oil and selenium, are expected to enhance reproductive function by reducing oxidative stress. This study aimed to investigate how these antioxidants affect the expression of fertility genes (StAR and PVRL3) in aging roosters. Forty-two Ross 308 broiler roosters, all over 50 weeks old, were used in a factorial experiment in a completely randomized design over 10 weeks. Treatments were: 1) Control diet, 2) Basic diet +100mg/kg rosemary essential oil, 3) +200mg/kg rosemary essential oil, 4) +0.3mg/kg selenium-enriched yeast, 5) +100mg/kg rosemary essential oil and 0.3mg/kg selenium-enriched yeast, and 6)+200mg/kg rosemary essential oil and 0.3mg/kg selenium-enriched yeast. At the end of the treatment, three samples of testicular tissue from each treatment were collected and stored at -80°C. The expression levels of the StAR and PVRL3 genes were measured using real-time quantitative PCR. The results indicated that rosemary oil did not significantly affect StAR gene expression. However, a dose of 200 mg/kg significantly reduced PVRL3 expression, whereas the 100 mg/kg dose did not show a significant effect. Selenium supplementation at a dosage of 0.3 mg/kg significantly increased the expression of the StAR and PVRL3 genes. Adding 100 mg/kg of rosemary along with 0.3 mg/kg of selenium significantly increased the expression of the PVRL-3 gene. However, when 200 mg of rosemary was added in the presence of selenium, a decrease in PVRL-3 gene expression was observed, and selenium did not prevent this decline. This suggests that using 200 mg of rosemary essential oil may be undesirable. Based on these results, adding 100 mg/kg of rosemary along with 0.3 mg of selenium-enriched yeast to the diet of older broiler breeder roosters is recommended.

Key words: Aged rooster, PVRL3, Rosemary, Selenium

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Refrences

Adeldust, H., Farzinpour, A., Farshad, A., Rostamzadeh, J., & Béjar, M. L. (2021). Effect of orally administrated letrozole on reproduction performance and gene expression of FOXJ1, LPR2 and PVRL3 in reproductive tract in aged roosters. *Theriogenology*, *161*, 131-139.

Adeldust, H., Farzinpour, A., Farshad, A., Vaziry, A., & Rostamzadeh, J. (2022). Effect of letrozole on reproductive indices of aged broiler breeder roosters. *Animal Production Research*, *11(1)*, 55-67.

Alahmadi, A. A., & Alahmadi, B. A. (2024). Rosemary leaf extract alleviates testicular impairment, oxidative stress, and apoptosis in streptozotocin-induced diabetic rats. *Tropical Journal of Pharmaceutical Research*, *23*(7), 1055-1061.

Ali, M. E., Zainhom, M. Y., Monir, A., Awad, A. A. E., & Al-Saeed, F. A. (2024). Dietary supplementation with rosemary essential oil improves genital characteristics, semen parameters and testosterone concentration in Barki rams. *The Journal of Basic and Applied Zoology*, *85*(1), 45.

Amin Altawash, A. S., ZarehShahneh, A., Moravej, H., Ansari, M., &Deldar, H. (2019). Improvement of testis histological parameters and relative expression of StAR gene in Chrysin-fed roosters. *Iranian Journal of Animal Science*, *50(3)*, 207-215.

Bansal, A. K., &Bilaspuri, G. S. (2011). Impacts of oxidative stress and antioxidants on semen functions. *Veterinary medicine international*, *2011*(1), 686137.

Borghei-Rad, S. M., Zeinoaldini, S., Zhandi, M., Moravej, H., & Ansari, M. (2017). Feeding rosemary leaves powder ameliorates rooster age-related subfertility. *Theriogenology*, *101*, 35-43.

Borrás-Linares, I., Stojanović, Z., Quirantes-Piné, R., Arráez-Román, D., Švarc-Gajić, J., Fernández-Gutiérrez, A., & Segura-Carretero, A. (2014). Rosmarinus officinalis leaves as a natural source of bioactive compounds. *International Journal of Molecular Sciences*, *15*(11), 20585-20606.

Bréque, C., Surai, P., &Brillard, J. P. (2003). Roles of antioxidants on prolonged storage of avian spermatozoa in vivo and in vitro. *Molecular Reproduction and Development: Incorporating Gamete Research*, *66*(3), 314-323.

Budai, C., Egerszegi, I., Olah, J., Javor, A., & Kovacs, A. (2014). The protective effect of antioxidants on liquid and frozen stored ram semen–review. *Scientific Papers Animal Science and Biotechnologies*, *47*(1), 46-46.

Cao, C., Zhang, H., Wang, K., & Li, X. (2020). Selenium-rich yeast mitigates aluminum-mediated testicular toxicity by blocking oxidative stress, inhibiting NO production, and disturbing ionic homeostasis. *Biological Trace Element Research*, *195*(1), 170-177.

El-Din, R. A. S., El-Shahat, A. E. R., Elmansy, R. A., EL-DIN, R. A. S., EL-SHAHAT, A. E., & ELMANSY, R. (2012). An electron microscopic study of the antifertility potential of rosemary (Rosmarinus officinalis L.) in male albino rats. *International Journal of Morphology*, *30*(2), 666-72.

Hadrup, N., &Ravn-Haren, G. (2021). Absorption, distribution, metabolism and excretion (ADME) of oral selenium from organic and inorganic sources: A review. *Journal of Trace Elements in Medicine and Biology*, *67*, 126801.

Heng, D., Zhang, T., Tian, Y., Yu, S., Liu, W., Xu, K., ...& Zhang, C. (2017). Effects of dietary soybean isoflavones (SI) on reproduction in the young breeder rooster. *Animal Reproduction Science*, *177*, 124-131.

Jahaabad, M. J., Ziaenejad, S., Ghayedi, A., Moradian, H., &SeyyediAbalvan, M. (2020). The effects of selenium nanoparticles enriched food on sperm quality and fertilization of rainbow trout (Oncorhynchus mykiss) male breeders. *Iranian Veterinary Journal*, *16(3)*, 106-115.

Khalil-Khalili, A. A., Zhandi, M., Zaghari, M., Mehrabani-Yeganeh, H., Yousefi, A. R., &Tavakoli-Alamooti, M. (2021). The effect of dietary organic selenium on reproductive performance of broiler breeder roosters under dexamethasone induced stress. *Theriogenology*, *161*, 16-25.

Khan, R. U. (2011). Antioxidants and poultry semen quality. *World's poultry science journal*, *67*(2), 297-308.

Lagares, M. A., Ecco, R., Martins, N. R. S., Lara, L. J. C., Rocha, J. S. R., Vilela, D. A. R., ... &Baião, N. C. (2017). Detecting reproductive system abnormalities of broiler breeder roosters at different ages. *Reproduction in Domestic Animals*, *52*(1), 67-75.

Lemoine, M., Mignon-Grasteau, S., Grasseau, I., Magistrini, M., &Blesbois, E. (2011). Ability of chicken spermatozoa to undergo acrosome reaction after liquid storage or cryopreservation. *Theriogenology*, *75*(1), 122-130.

Liu, L., Wu, C., Chen, D., Yu, B., Huang, Z., Luo, Y., ...& He, J. (2020). Selenium-enriched yeast alleviates oxidative stress-induced intestinal mucosa disruption in weaned pigs. *Oxidative Medicine and Cellular Longevity*, *2020*(1), 5490743.

Maneesh, M., &Jayalekshmi, H. (2006). Role of reactive oxygen species and antioxidants on pathophysiology of male reproduction. *Indian Journal of Clinical Biochemistry*, *21*, 80-89.

MansouriTorghabeh, F., Rostamzadeh, P., Davoudi, S., Keivan, M., &Shokri-Asl, V. (2022). Effects of Rosmarinus officinalis on orchitis following spermatic cord torsion-detorsion in male mice with emphasis on anti-inflammatory and antioxidant properties. *Andrologia*, *54*(1), e14252.

McBride, J. A., & Coward, R. M. (2016). Recovery of spermatogenesis following testosterone replacement therapy or anabolic-androgenic steroid use. *Asian journal of andrology*, *18*(3), 373-380.

Miazi, O. F., Miah, G., Miazi, M. M., Uddin, M. M., Hassan, M. M., &Faridahsan, M. (2012). Fertility and hatchability of Fayoumi and Sonali chicks. *Scholarly Journal of Agricultural Science*, *2*(5), 83-86.

Mosavi, S.T., BeigiNassiri, M.T., Roshanfekr, H.R., &Nazari, M. (2022). The effect of Vitexagnusecastus fruits powder on oviduct markers gene expression of laying hens. *Research on Animal Production*, *13(38)*, 138-146.

Nazari, M., Ghorbani, M. R., BeigiNassiri, M. T., Fathimoghadam, N., Sabahi, R., &Mosavi, S. T. (2023). The effects of Chaste-berry fruits on hypothalamic-pituitary-ovarian markers gene expression and immune response of laying hens: Phytoestrogens in Chaste-berry are ERβ-selective. *Iranian Veterinary Journal*, *19*(1), 61-71.

Nazari, M., &Rostami, S. (2024). Effect of myrtle essential oil (MEO) on gene expression of cytokine profiles in the small intestine of broiler chicken. *Iranian Veterinary Journal*, *19*(4), 174-184.

Pappas, A. C., Zoidis, E., Surai, P. F., &Zervas, G. (2008). Selenoproteins and maternal nutrition. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *151*(4), 361-372.

Parlat, S. S., Yıldız, A. Ö., Cufadar, Y., &Olgun, O. (2005). Effect of thyme essential oil (Origanum Vulgare L.) supplementation on performance of Japanese quail exposed to experimental Aflatoxicosis. *Selcuk Journal of Agriculture and Food Sciences*, *19*(36), 1-6.

Pfaffl, M. W. (2004). Quantification strategies in real-time PCR. *AZ of quantitative PCR*, *1*, 89-113.

Qazi, I. H., Angel, C., Yang, H., Zoidis, E., Pan, B., Wu, Z., ... & Zhou, G. (2019). Role of selenium and selenoproteins in male reproductive function: a review of past and present evidences. *Antioxidants*, *8*(8), 268.

Rabieh, M., Rooshanfekr, H., Nazari, M., &Ghorbani, M.R. (2020). Gene expression of antioxidant enzymes fed wild pistachio (Pistachio atlantica), purslane (portulacaoleracea) extract and vitamin E under in broiler chickens under heat stress condition. *Iranian Veterinary Journal*, *17(2)*, 51-60.

Rostami, H., Seidavi, A., Dadashbeiki, M., Asadpour, Y., Simões, J., Laudadio, V., ...&Tufarelli, V. (2017). Oxidative stability of chilled broiler breast meat as affected by dietary supplementation with rosemary (Rosmarinus officinalis L.) powder and vitamin E. *Food Science & Nutrition*, *5*(4), 904-910.

Sabahi, R., Nazari, M., BeigiNassiri, M. T., &ghorbani, M. R. (2020). The Effect of VitexAgnuseCastus fruit powder on hypothalamic GnRH gene expression in laying hens. *Research on Animal Production*, *11 (30)*, 92-100.

Samo, S. P., Malhi, M., Kachiwal, A. B., Gadahi, J. A., Parveen, F., Kalhoro, N. H., & Lei, Y. (2020). Supranutritional selenium level minimizes high concentrate diet-induced epithelial injury by alleviating oxidative stress and apoptosis in colon of goat. *BMC Veterinary Research*, *16*, 1-10.

Schrauzer, G. N. (2001). Nutritional selenium supplements: product types, quality, and safety. *Journal of the American College of Nutrition*, *20*(1), 1-4.

Shahraki Mojahed, L., Nazifi, S., Saeb, M., & Eskandarzade, N. (2024). The long-term effect of hydro-alcoholic Aloe vera extract on serum levels of LH, FSH, inhibin B, anti-mullerian hormone, and testosterone in adult male rats. *Iranian Veterinary Journal*, *19*(4), 76-82.

Shi, L. G., Yang, R. J., Yue, W. B., Xun, W. J., Zhang, C. X., Ren, Y. S., & Lei, F. L. (2010). Effect of elemental nano-selenium on semen quality, glutathione peroxidase activity, and testis ultrastructure in male Boer goats. *Animal reproduction science*, 118(2-4), 248-254.

Stocco, D. M., Clark, B. J., Reinhart, A. J., Williams, S. C., Dyson, M., Dassi, B., ...& Orly, J. (2001). Elements involved in the regulation of the StAR gene. *Molecular and cellular endocrinology*, *177*(1-2), 55-59.

Sun, Y., Xue, F., Li, Y., Fu, L., Bai, H., Ma, H., ...& Chen, J. (2019). Differences in semen quality, testicular histomorphology, fertility, reproductive hormone levels, and expression of candidate genes according to sperm motility in Beijing-You chickens. *Poultry science*, *98*(9), 4182-4189.

Surai, P. F., Blesbois, E., Grasseau, I., Chalah, T., Brillard, J. P., Wishart, G. J., ... & Sparks, N. H. C. (1998). Fatty acid composition, glutathione peroxidase and superoxide dismutase activity and total antioxidant activity of avian semen. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *120*(3), 527-533.

Xiong, N., Liu, S., Hu, W., Liu, Y., Ding, X., Xu, D., ... & Wu, B. (2025). Selenium yeast alleviates diquat-induced oxidative stress and testicular damage in roosters. *Animal Reproduction Science*, 273, 107760.

Yang, X., Yang, C., Tang, D., Yu, Q., & Zhang, L. (2022). Effects of dietary supplementation with selenium yeast and jujube powder on mitochondrial oxidative damage and apoptosis of chicken. *Poultry science*, *101*(10), 102072.

Zachara, B. A. (1992). Mammalian selenoproteins. *Journal of trace elements and electrolytes in health and disease*, *6*(3), 137-151.

Zarghi, H., Golian, A., &Kermanshahi, H. (2015). The effect of rosemary hydro-alcoholic (Rosmarinus Officinalis L.) extract on performance and egg quality in laying hens. *Iranian Journal of Animal Science*, *46*(1), 1-8.

Zhandi, M., Talebnia-Chalanbar, A., Towhidi, A., Sharafi, M., Yousefi, A. R., & Hussaini, S. M. H. (2020). The effect of zinc oxide on rooster semen cryopreservation. *British poultry science*, *61*(2), 188-194.